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(54) Title: METHODS AND COMPOSITIONS FOR IDENTIFYING OSTEOGENIC AGENTS

(57) Abstract

Methods and compositions for identifying osteogenic agents are disclosed, wherein a bone morphogenetic protein promoter is utilized in an assay system to modulate the production of an assayable product of a reporter gene.

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METHODS AND COMPOSITIONS FOR IDENTIFYING OSTEOGENIC AGENTS

Technical Field

The present invention relates to assay techniques for identifying agents which modulate bone growth.

Background of the Invention

Although there is a great deal of information available on the factors which influence the breakdown and resorption of bone, information on growth factors which stimulate the formation on growth factors which stimulate the formation of new bone is more limited. Investigators have searched for sources of such activities and have found that bone tissue itself is a storehouse for factors which have the capacity for stimulating bone cells. Thus, extracts of bovine tissue obtained from slaughterhouses contain not only structural proteins which are responsible for maintaining the structural integrity of bone, but also biologically active bone growth factors which can stimulate bone cells to proliferate. Among these latter factors are transforming growth factor β, the heparin-binding growth factors (acidic and basic fibroblast growth factor), the insulin-like growth factors (insulin-like growth factor I and insulin-like growth factor II) and a recently described family of proteins called bone morphogenetic proteins (BMPs). All of these growth factors have effects on other types of cells as well as on bone cells.

The BMPs are novel factors in the extended transforming growth factor β family. They were first identified in extracts of demineralized bone (Urist 1965, Wozney *et al.*, 1988). Recombinant BMP-2 and BMP-4 can induce new bone formation when they are injected locally into the subcutaneous tissues of rats (Wozney 1992, Wozney & Rosen 1993). These factors are expressed by normal osteoblasts as they differentiate, and have been shown to stimulate osteoblast differentiation and bone nodule formation *in vitro* as well as bone formation *in vivo* (Harris *et al.*, 1994). This latter property suggests potential usefulness as therapeutic agents in diseases which result in bone loss.

The cells which are responsible for forming bone are osteoblasts. As osteoblasts differentiate from precursors to mature bone-forming cells, they express and secrete a number of the structural proteins of the bone matrix including Type-1 collagen, osteocalcin, osteopontin and alkaline phosphates (Stein *et al.*, 1990, Harris *et al.*, 1994). They also

synthesize a number of growth regulatory peptides which are stored in the bone matrix and are presumably responsible for normal bone formation. These growth regulatory peptides include the BMPs (Harris *et al*, 1994). In studies of primary cultures of fetal rat calvarial osteoblasts, BMPs 1, 2, 3, 4, and 6 are expressed by cultured cells prior to the formation of mineralized bone nodules (Harris *et al*, 1994). Expression of the BMPs coincides with expression of alkaline phosphatase, osteocalcin and osteopontin.

Although the BMPs have powerful effects to stimulate bone formation *in vitro* and *in vivo*, there are disadvantages to their use as therapeutic agents to enhance bone healing. Receptors for the bone morphogenetic proteins have been identified in many tissues, and the BMPs themselves are expressed in a large variety of tissues in specific temporal and spatial patterns. This suggests that they may have effects on many tissues other than bone, potentially limiting their usefulness a therapeutic agents when administered systematically. Moreover, since they are peptides, they would have to be administered by injection. These disadvantages are severe limitations to the development of BMPs as therapeutic agents.

It is an object of the present invention to overcome the limitations inherent in known osteogenic agents by providing a method to identify potential drugs which would stimulate production of BMPs locally in bone.

Prior Art

Sequence data on small fragments of the 5'-flanking region of the BMP-4 gene have been published (Chen *et al*, 1993; Kurihara *et al*, 1993), but the promoter has not been previously functionally identified or isolated.

Disclosure of the Invention

A cell-based assay technique for identifying and evaluating compounds which stimulate the growth of bone is provided, comprising culturing a host cell line comprising an expression vector comprising a DNA sequence encoding a promoter region of at least one bone morphogenetic protein, operatively linked to a reporter gene encoding an assayable product under conditions which permit expression of said assayable product, contacting the cultured cell line with at least one compound suspected of possessing osteogenic activity, and identifying osteogenic agents by their ability to modulate the expression of the reporter gene and thereby increase the production of the assayable product.

This assay technique specifically identifies osteogenic agents which stimulate bone cells to produce bone growth factors in the bone morphogenetic protein family. These osteogenic agents display the capacity to increase the activity of the promoters of genes of members of the BMP family and other bone growth factors normally produced by *e.g.* bone cells.

Also provided in accordance with the present invention are isolated DNA sequences encoding a promoter region of at least one bone morphogenetic protein, and a system for identifying osteogenic agents comprising an expression vector comprising such promoter sequences operatively linked to a reporter gene encoding an assayable product, and means for detecting the assayable product produced a response to exposure to an osteogenic compound.

Brief Description of the Drawings

Figure 1A graphically depicts a restriction enzyme map of mouse genomic BMP-4 and a diagram of two transcripts. The mouse BMP-4 gene transcription unit is ~7kb and contains 2 coding exons (closed boxes) and 3 non-encoding exons, labeled exons 1A, 1B and 2. This 19kb clone has an ~6kb 5′-flanking region and an ~7kb 3′-flanking region. The diagram shows approximately 2.4kb of the 5′-flanking region, and a small region of the 3′-flanking region. The lower panel shows two alternative transcripts of BMP-4. Both have the same exons 2, 3 and 4 but a different exon 1. Transcript A has exon 1A and transcript B has exon 1B whose size was estimated according to RT-PCR and primer extension analysis in FRC cells;

Figure 1B depicts the DNA sequence of selected portions of mouse genomic BMP-4 (SEQ. ID NO. 1) and the predicted amino acid sequences of the identified coding exons (SEQ. ID NO. 2). The numbers on the right show the position of the nucleotide sequence and the bold numbers indicate the location of the amino acid sequence of the coding region. Most of the coding sequence is in exon 4. The end of the transcription unit was estimated based on a 1.8kb transcript. Primer 1 in exon 1A was used in RT-PCR analysis with Primer 3 in exon 3. Primer 2 in exon 1B was used in RT-PCR analysis with Primer 3. Primer B1 and B2 were used in primer extension reactions;

Figure 1C portrays the sequence of the BMP-4 exon 1A 5′-flanking region and potential response elements in the mouse BMP-4 1A promoter (SEQ. ID NO. 3). The

sequences of 2688 bp of the mouse BMP-4 gene are shown. Nucleotides are numbered on the left with +1 corresponding to the major transcription start site of the 1A promoter. The response elements of DR-1A Proximal and DR-1A Distal oligonucleotides are indicated. The other potential response DNA elements in the boxes are p53, RB (retinoblastoma), SP-1, AP-1, and AP-2. Primer A, indicated by the line above the DNA sequence at +114 to +96, was used for primer extension analysis of exon 1A-containing transcripts;

Figure 2 depicts the results of a primer extension assay. Total RNAs prepared from FRC cells (on the left frame) and mouse embryo 9.5 days (on the right) were used with primer A or the complement of primer 2. Two major extended fragments, 67 and 115 bp, indicated a lane A were obtained from primer A. Two 1B primers, primer B1 and primer B2, also gave negative results with both FRC and mouse embryo total RNA as template. Transcript B is not detectable with this assay. By RT-PCR, transcript B can be detected and quantified;

Figure 3A is a photographic representation of gel electrophoresis of 1A-3 and 1B-3 RT-PCR products of the BMP-4 gene. RT-PCR was performed with two pairs of primers using FRC cell poly A$^+$ mRNA as the template. The products were verified by the DNA sequence;

Figure 3B is a schematic diagram of spliced BMP-4 RT-PCR products with 1A and 1B exons in FRC cells. RT-PCR was performed with two pairs of primers using FRC cell poly A$^+$ mRNA as the template. The diagram shows where the primers are located in the BMP-4 genomic DNA. RT-PCR product 1A-2-3 which contains exon 1A, exon 2 and the 5′ region of exon 3, was produced with primer 1 and primer 3. Primer 2 and primer 3 generated two RT-PCR products with the exon 1B-2-3 pattern. The heterogeneity in size of exon 1B is indicated. The 1A promoter is predominantly utilized in bone cells;

Figure 4A provides a map of the BMP-4 1A 5′ -flanking-CAT plasmid and promoter activity in FRC cells. The 2.6kb <u>Eco</u>RI and <u>Xba</u> fragment, 1.3 kb <u>Pst</u> fragment, 0.5kb <u>Sph</u>I and <u>Pst</u> fragment, and 0.25kb PCR fragment were inserted into pBLCAT3. The closed box indicates the non-coding exon 1A. The CAT box represents the CAT reporter gene. The values represent percentages of CAT activity expressed by pCAT-2.6 set at 100%. The values represent the average of four independent assays;

Figure 4B provides an autoradiogram of CAT assays using FRC cells transfected with BMP-4 1A 5′-flanking-CAT plasmids identified in Figure 4A;

Figure 5 portrays the nucleotid sequence of the mouse BMP-2 gene 5′ -flanking region from -2736 to +139 (SEQ. ID NO. 4). The transcription start site is denoted by +1;

Figure 6A depicts an autoradiogram showing products of a primer extension assay for determination of the transcription start site of the BMP2 gene, separated on a 8% denaturing urea-polyacrylamide gel, in which Lane 1: Total RNA from fetal rat calvarial osteoblast cells, and Lane 2: Control lane with 10μg of yeast tRNA. All RNA samples were primed with a ^{32}p-labeled oligonucleotide from exon 1 to the mouser BMP2 gene, as indicated in Figure 6B. Lane M: ^{32}p-labeled MspI digested λ phage DNA, containing DNA fragments spanning from 623 bp to 15 bp (size marker);

Figure 6B provides a schematic representation of the primer extension assay. The primer used is a 18mer synthetic oligonucleotide, 5′-CCCGGCAAGTTCAAGAAG-3′ (SEQ. ID NO. 5);

Figure 7 provides a diagram of selected BMP-2 promoter - luciferase reporter constructs. BMP-2 5′ -flanking sequences are designated by hatched boxes (□) and luciferase cDNA is designated by the filled box (■). Base +114 denotes the 3′ end of the BMP-2 gene in all the constructs;

Figure 8 displays the luciferase enzyme activity for the BMP-2 gene-LUC constructs (shown in Figure 7) transfected in primary fetal rat calvarial osteoblasts (A), HeLa cells (B) and ROS 17/2.8 osteoblasts (C). The luciferase activity has been normalized to β-galactosidase activity in the cell lysates;

Figure 9A-F depicts the DNA sequence of the mouse BMP-2 promoter and gene (SEQ. ID NO. 6); and

Figure 10A-D depicts the DNA sequence of the mouse BMP-4 promoter and gene (SEQ. ID NO. 7).

Figure 11 depicts the resequencing of the BMP-2 5′ flanking region.

Detailed Description of the Preferred Embodiments

A cell-based assay technique for identifying and evaluating compounds which stimulate the growth of bone is provided, comprising culturing a host cell line comprising an expression vector comprising a DNA sequence encoding a promoter region of at least one bone morphogenetic protein operatively linked to a reporter gene encoding an assayable product under conditions which permit expression of said assayable product, contacting the cultured cell line with at least one compound suspected of possessing osteogenic activity, and identifying osteogenic agents by their ability to modulate the expression of the reporter gene and thereby increase the production of the assayable product.

The present invention is distinguished from other techniques for identifying bone-active compounds, as it specifically identifies chemical compounds, agents, factors or other substances which stimulate bone cells to produce the bone growth factors in the bone morphogenetic protein (BMP) family (hereinafter "osteogenic agents"). These osteogenic agents are identified by their capacity to increase the activity of the promoters of genes of members of the BMP family and other bone growth factors which are normally produced by bone cells, and other cells including cartilage cells, tumor cells and prostatic cells. When patients are treated with such chemical compounds, the relevant BMP will be produced by bone cells and then be available locally in bone to enhance bone growth or bone healing. Such compounds identified by this assay technique will be used for the treatment of osteoporosis, segmental bone defects, fracture repair, prosthesis fixation or any disease associated with bone loss.

Compounds that inhibit bone morphogenetic protein expression in bone or cartilage may also be useful in clinical situations of excess bone formation which occurs in such diseases as osteoblastic metastases or osteosclerosis of any cause. Such compounds can also be identified in accordance with the present invention.

Also provided in accordance with the present invention are isolated DNA sequences encoding a promoter region of at least one bone morphogenetic protein, and a system for identifying osteogenic agents comprising an expression vector comprising such promoter sequences operatively linked to a reporter gene encoding an assayable product, and means for detecting the assayable product produced in response to exposure to an osteogenic compound.

The promoters of the genes for BMP-4 and BMP-2 are complex promoters which can be linked to reporter genes, such as *e.g.* the firefly luciferase gene. When the hybrid genes (for example, bone cell BMP-4 promoter or bone cell BMP-2 promoter and firefly luciferases, chloramphenicol acetyl transferase (CAT) cDNAs, or cDNA's for other reporter genes such as β-galactosidase, green fluorescent protein, human growth hormone, alkaline phosphatase, β-glucuronidase, and the like) are transfected into bone cells, osteogenic agents which activate the BMP-4 or BMP-2 promoters can be identified by their capacity *in vitro* to increase luciferase activity in cell lysates after cell culture with the agent.

Sequence data on small fragments of the 5′-flanking region of the BMP-4 gene have been published (Chen *et al*, 1993; Kurihara *et al*, 1993), but the promoter has not been previously identified or isolated, and methods for regulating transcription have not been shown. The present invention isolates the promoters for the BMP genes and utilizes these promoters in cultured bone cells so that agents could be identified which specifically increase BMP-2 or BMP-4 production locally in bone. Since it is known that the BMPs are produced by bone cells, a method for enhancing their production specifically in bone should avoid systemic toxicity. This benefit is obtained by utilizing the unique tissue specific promoters for the BMPs which are provided herein, and then using these gene promoters to identify agents which enhance their activity in bone cells.

By utilizing the disclosure provided herein, other promoters can be obtained from additional bone morphogenetic proteins such as BMP-3, BMP-5, BMP-6, and BMP-7, to provide comparable benefits to the promoters herein specifically described.

In addition, the present invention contemplates the use of promoters from additional growth factors in osteoblastic cells. Included are additional bone morphogenetic proteins, as well as fibroblast growth factors (*e.g.* FGF-1, FGF-2, and FGF-7), transforming growth factors β-1, β-2, and β-3, insulin-like growth factor-1, insulin-like growth factor-2, platelet-derived growth factor, and the like. Such promoters will readily be utilized in the present invention to provide comparable benefits.

The cells which can be utilized in the present invention include primary cultures of fetal rat calvarial osteoblasts, established bone cell lines available commercially (MC3T3-E1 cells, MG-63 cells, U2OS cells, UMR106 cells, ROS 17/2.8 cells, SaOS2 cells, and the like

as provided in the catalog from the American Type Culture Collection (ATCC)), and bone cell lines established from transgenic mice, as well as other cell lines capable of serving as hosts for the present vectors and systems. In addition, a number of tumor cell lines also express BMPs, including the prostate cancer cell lines PC3, LNCAP, and DUI145, as well as the human cancer cell line HeLa. Thus, any of a number of cell lines will find use in the present invention and the choice of an appropriate cell line will be a matter of choice for a particular embodiment.

The following examples serve to illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

EXPERIMENTAL

In the experimental disclosure which follows, the following abbreviations apply: eq (equivalents); M (Molar); mM (millimolar); μM (micromolar); N (Normal); mol (moles); mmol (millimoles); μmol (micromoles); nmol (nanomoles); kg (kilograms); gm (grams); mg (milligrams); μg (micrograms); ng (nanograms); L (liters); ml (milliliters); μl (microliters); vol (volumes); and °C (degrees Centigrade).

Example 1: DESCRIPTION AND CHARACTERIZATION OF MURINE BMP-4 GENE PROMOTER

(a) Library Screening, Cloning and Sequencing of Gene

A mouse genomic lambda fix II spleen library (Stratagene, La Jolla, CA) was screened with a mouse embryo BMP-4 cDNA kindly provided by Dr. B.L.M. Hogan (Vanderbilt University School of Medicine, Nashville, TN). The probe was labeled with [α-^{32}P]dCTP using a random-primer labeling kit from Boehringer-Mannheim (Indianapolis, IN). Plaque lift filters were hybridized overnight in 6X SSC, 5X Denhardt's. 0.5% SDS containing 200μg/ml sonicated salmon sperm DNA, 10μg/ml Poly A and 10μg/ml t-RNA at 68° C. The filters were washed at 55° C for 20 min, twice in 2X SSC, 0.1% SDS buffer, once in 0.5X SSC, 0.1% SDS. The isolated phage DNA clones were analyzed according to standard procedures (Sambrook *et al.*, 1989).

Fragments from positive clones were subcloned into pBluescrpt vectors (Stratagene, La Jolla, CA) and sequenced in both directions using the Sequenase

dideoxynucleotide chain termination sequencing kit (U.S. Biochemical Corp., Cleveland, OH).

Three clones were isolated from $2x10^6$ plaques of mouse spleen 129 genomic library using full length coding region mouse embryo BMP-4 cDNA probe (B. Hogan, Vanderbilt University, Nashville, TN). One 19kb clone contained 5 exons and ~6kb 5′-flanking region and a ~7kb 3′-flanking region, as shown in Figure 1A. The 7kb transcription unit and the 5′-flanking region of the mouse BMP-4 gene were sequenced (Figure 10).

The nucleotide sequence of selected portions of mouse BMP-4 and the deduced amino acid sequence of the coding exons (408 residues; SEQ. ID NO. 2) is shown in Figure 1B. Primers used in the RT-PCR experiments described below are indicated in this Figure.

Figure 1C shows the DNA sequence of 2372bp of the 5′-flanking region and the candidate DNA response elements upstream of exon 1A. Primers used in primer extensions are also shown in Figures 1B and 1C.

(b) Primer Extension Mapping of the Transcriptional Start-Site of the Mouse BMP-4 Gene

The transcriptional start-sites were mapped by primer extension using the synthetic oligonucleotide primer A 5′-CGGATGCCGAACTCACCTA-3′ (SEQ. ID NO. 8), corresponding to the complement of nucleotides +114 to +96 in the exon 1A sequence and the oligonucleotide primer B1 5'-CTACAAACCCGAGAACAG-3' (SEQ. ID NO. 9), corresponding to the complement of nucleotides +30 to +13 of the exon 1B sequence. Total RNA from fetal rat calvarial (FRC) cells and 9.5 day mouse embryo (gift of B. Hogan, Vanderbilt University) was used with both primers. The primer extension assay was carried out using the primer extension kit from Promega (Madison, WI). The annealing reactions were, however, carried out at 60°C in a water bath for 1 hr. The products were then electrophoresed on 8% denaturing-urea polyacrylamide gels and autoradiographed.

One additional oligonucleotide primer B2 5′-CCCGGCACGAAAGGAGAC-3′ (SEQ. ID NO. 10), corresponding to the complement of nucleotide sequence +69 to +52 of exon 1B, was also utilized in primer extension reactions with FRC and mouse embryo RNAs.

1. Evidence for utilization of two alternate exon 1 sequences for the BMP-4 gene.

Several BMP-4 cDNAs were sequenced from prostate cancer cell in PC-3 and from primary FRC cells. Four independent FRC cell BMP-4 cDNAs all contained exon 1A. However, the human prostate carcinoma cell line (PC-3) cDNA contained an apparently unique exon 1B sequence spliced to exon 2 (Chem *et al*, 1993). A doubt-stranded oligonucleotide roble (70bp) to exon 1B was synthesized based on the human PC-3 exon 1B sequence. This exon 1B probe was then used to identify the exon 1B region in the mouse genomic BMP-4 clone. The candidate exon 1B is 1696bp downstream from the 3' end of exon 1A.

2. Primer extension analysis

Primer extension analysis was performed to map the mouse BMP-4 gene transcription start sites. Primer A, an oligonucleotide from exon 1A, was used and two oligonucleotides from exon 1B. Total RNA was utilized both from mouse embryo and FRC cells. As shown in Figure 2, a major extended fragment from primer A was obtained in both mouse embryo and FRC cell total RNAs, which migrates at 115bp. The extended 5'-end of the 115bp fragment represents the major transcription start site for 1A-containing transcripts. The site of this 5' non-coding exon 1A is 306bp. A major extended fragment from the complement of primer B1 (exon 1B) was not detected using both mouse embryo and FRC cell total RNAs. One other primer from exon 1B also gave negative results, suggesting that in 9.5 day mouse embryo and FRC cells, the exon 1B-containing transcripts were not detectable, which suggests that transcripts containing exon 1B are less abundant in these cells and tissues than transcripts containing exon 1A. All primer extensions were carried out after annealing of primers at high stringency. Lower stringency annealing with 1B primers gave extended products not associated with BMP-4 mRNA.

(c) BMP-4 Gene 5' Flanking Region for Exon 1A and 1B Transcripts.

Four FRC BMP-4 cDNA were sequenced and found to contain exon 1A sequences spliced to exon 2. The human U20S BMP-4 cDNA sequence also contains exon 1A (Wozney *et al*, 1988). This suggests the BMP-4 gene sequences upstream or exon 1A are used primarily in bone cells.

To test whether the BMP-4 1B promoter is utilized at all in FRC cells, oligonucleotide primers were designed to ascertain whether spliced 1B-2-3 exon products

and 1A-2-3 exon (control) products could be obtained by more sensitive RT-PCR technique using FRC poly (A^+)-RNA. The 3' primer was in exon 3 (Figure 1B - Primer 3) and the 5' primers were either in exon 1A (primer 1) or exon 1B (primer 2).

The RT-PCR products were cloned and sequenced. A photograph and diagram of the products obtained are presented in Figure 3A and B. Both 1A-2-3 and 1B-2-3 products were obtained. The results indicate FRC osteoblasts produce transcripts with either 1A exon or a 1B exon, but not both. This suggests that the intron region between 1A and 1B exons could contain regulatory response elements under certain conditions. Of the 1B-2-3 RT-PCR products obtained from FRC osteoblasts, two products were obtained with different 3′ splice sites for the exon 1B. By comparison with the genomic DNA, both 3′ ends of the two exon 1Bs have reasonable 5′ splice consensus sequences, consistent with an alternate splicing pattern obtained for the 1B-2-3 RT-FCR products. Most importantly, no 1A-1B-2-3 RT-PCR splice products of the BMP-4 gene were obtained. Thus, 1B does not appear to be alternatively spliced 5′-non-encoding exon. By quantitative RT-PCR, it was shown that 1A transcripts are 10 to 15X more abundant in primary bone cells.

The technique of performing RT-PCR will be described. First-strand cDNA was synthesized from 1μg FRC cell poly (A^+)-RNA with an 18mer dT primer using Superscript™ reverse transcriptase (Gibco BRL) in a total volume of 20μl. The cDNA was then used as a template for PCR with two sets of synthesized primers. As shown in Figure 1B, primer 1 (5′-GAAGGCAAGAGCGCGAGG-3) (SEQ. ID No. 11), corresponding to a 3′ region of exon 1A and primer 3 (5′-CCGGTCTCAGGTATCA-3') (SEQ. ID No. 12), corresponding to a 5' region of exon 3 were used to generate exon 1A-2-3 spliced PCR product. Primer 2 (5′-CAGGCGGAAAGCTGTTC-3') (SEQ. ID NO. 13), corresponding to a 3′ region (+2 to +18) of exon 1B, and primer 3 were used to generate exon 1B-2-3 spliced PCR products. GeneAmp PCR kit was used according to the manufacturer's procedure (Perkin-Elmer/Cetus, Norwalk, CT). Each cycle consisted of a denaturation step (94°C for 1 min), an annealing step (59°C for 2 min) and an elongation step (72°C for 1 min). The PCR products were analysed by agarose gel electrophoresis for size determination. The products were subcloned into pCR II vector using TA cloning kit (InVitrogen, San Diego, CA). The inserts were sequenced in both directions with a sequencing kit from U.S. Biochemical (Cleveland, OH).

Northern analysis demonstrated that the single 1.8kb BMP-4 transcript detected in FRC cells during bone cell differentiation hybridizes to both a pure 1A exon probe and a 2-4 exons probe. The ratio of the 1A to 2-4 signal is constant through the changing levels of BMP-4 expression during differentiation. Using a 1B exon probe no detectable hybridization to the BMP-4 exon 2-4 1.8kb signal was observed. This again indicates that 1A containing transcripts predominate in bone cells, although 1B transcripts can be detected by the more sensitive PCR method. By quantitative PCR it was shown that 1A transcripts are 10-15X more abundant than 1B in FRC cells.

(d) BMP-4 Promoter 1A Plasmid Construction and Transfection, and Detection of Promoter Activity in Osteoblasts.

Three BMP-4 1A promoter/plasmids were constructed by excising fragments from the 5' flanking region of the mouse BMP-4 gene and cloning into pBL3CAT expression vectors (Luckow and Schutz, 1987). The pCAT-2.6 plasmid was the pBLCAT3 vector with a 2.6kb EcoR1 and Xba I fragment (-2372/+258) of the BMP-4 gene. The pCAT-1.3 plasmid was similarly generated from a 1.3kb Pst fragment (-1144/+212). The pCAT-0.5 plasmid was made from a 0.5kb SphI and Pst fragment (-260/+212). Both the pCAT-1.3 and the pCAT-0.5 plasmids have 212bp of exon 1A non-coding region. An additional promoter/plasmid was created from a PCR amplified product, corresponding to the 240bp sequence between nucleotides -25 and +212, and referred to as the pCAT-0.24. The amplified fragment was first cloned into pCR II vector using TA cloning kit (InVitrogen, San Diego, CA) and then the fragment was released with Hind III and Xho I, and relegated into pBL3CAT. Correct orientation of all inserts with respect to the CAT vector was verified by DNA sequencing.

The cells used for transient transfection studies were isolated from 19 day-old fetal rat calvariae by sequential digestion with trypsin and collagenase, as described by Bellows *et al*, (1986) and Harris *et al*, (1994). In brief, the calvarial bone were surgically removed and cleaned by washing in α minimal essential media (αMEM) containing 10% V/V fetal calf serum (FCS) and antibiotics. The bones were minced with scissors and were transferred to 35mm tissue culture dish containing 5ml of sterile bacterial collagenase (0.1%) and trypsin l (0.05%). This was then incubated at 37°C for 20 min. The cells released at this time were collected and immediately mixed with an equal volume of FCS to inactivate trypsin. This procedure is repeated 6 times to release cells at 20 min intervals.

Cells released from 3rd, 4th, 5th and 6th digestion (enriched for osteoblasts) were combined and the cells are collected by centrifugation at 40 Xg for 5 min. The cells were then plated in αMEM containing 10% FCS and antibiotics and were grown to confluency (2-3 days). At this stage the cells were plated for transfection in 60mm tissue culture dishes at a cell density of 5 x 10^5 cells per dish. These primary osteoblast cultures are capable of self-organizing into bone-like structure in prolonged cultures (Bellows *et al*, 1986; Harris *et al*, 1994). HeLa, ROS 17/2.8, and CV-1 cells were purchased from the ATCC.

The isolated FRC cells, enriched for the osteoblast phenotype, were used as recipient cells for transient transfection assays. BMP-4 mRNA is modulated in these cells in a transient fashion during prolonged cultured (Harris *et al*, 1994b). The technique of electroporation was used for DNA transfection (Potter, 1988; van den Hoff *et al*, 1992). After electroporation, the cells were divided into aliquots, replated in 100mm diameter culture dishes and cultured for 48 hours in modified Eagle's minimal essential media (MEM, GIBCO, Grand Island, NY) with 10% fetal calf serum (FCS). The extracts were assayed for CAT actively according to the method described by Gorman (1988) and CAT activity was normalized by β-galactosidase assay according to the method of Rouet *et al* (1992).

After 48 hrs of transfections with various BMP-4-CAT reporter gene plasmid constructs, the cells were harvested and the CAT activity was determined. As indicated in Figure 4A and 4B, pCAT-0.24 plasmid (-25/+212) has little CAT activity. This plasmid contains -25 to +212 of the 5′ non-coding exon 1A and was 3-fold lower that the parent pBL3CAT plasmid. The pCAT-0.5 (-260/+212), pCAT-1,3 (-1144/+212), and pCAT-2.6 (-2372/+258) showed progressive increasing CAT activity when transfected into FRC cells. These data are shown in Figure 4B. With pCAT-0.5 (-260/+212) there is a 10-fold increase in CAT activity relative to pCAT-0.24 (-25/+212). pCAT-1.3 (-1144/+212) shows a further 6-fold increase and pCAT-2.6 (-2372/+258) shows further 2-fold change over pCAT-1.3 (-1144/+212). Thus the net increase in CAT activity between the pCAT-0.24 (+257/+212) and the pCAT-2.6 (-2372/+258) in FRC cells is approximately 100-fold.

Example 2: DESCRIPTION AND CHARACTERIZATION OF MURINE BMP-2 GENE PROMOTER

(a) Cloning of Mouse BMP-2 Genomic DNA.

Genomic clones of the mouse BMP-2 gene were isolated in order to determine the transcriptional regulation of the BMP-2 gene in primary osteoblasts. 5×10^6 plaques were screened from a mouse genomic library, B6/CBA, (purchased from Stratagene, San Diego, CA) using BMP-2 cDNA as probe. The BMP-2 cDNA clone was isolated from a cDNA library of PC3 prostate cancer cells (Harris *et al*, 1994). The human BMP-2 probe was a 1.1kb SmaI fragment containing most of the coding region.

The BmP-2 genomic clones were sequenced by dideoxy chain termination method (Sanger *et al*, 1977), using deoxyadenosine 5′-[α[^{35}S]thio] triphosphate and Sequenase (United States Biochemical, Cleveland, OH). All fragments were sequenced at least twice and overlaps were established using the appropriate oligonucleotie primer. Primers were prepared on an Applied Biosystems Model 392 DNA Synthesizer. Approximately 16kb of one of these BMP-2 clones was completely sequenced (Figure 9). Analysis of this sequence showed that the mouse BMP-2 gene contains one encoding and two coding exons (Feng *et al*, 1994). Analysis of the 5' flanking sequence showed that the BMP-2 gene does not contain typical TATA oar CAAT boxes. However, a number of putative response elements and transcription factor recognition sequences were identified upstream of exon 1 (Figure 5). The 5′-flanking region is GC rich with several SP-1, AP-1 P53, E-box, homeobox, and AP-2 candidate DNA binding elements.

(b) Analysis of Transcription Start Site for BMP-2 Gene.

The transcription start sites for the BMP-2 gene were identified using the primer extension technique. Primer extension was carried out as described (Hall *et al.*, 1993). The primer used was a ^{32}p-labeled 18 mer oligonucleotide 5′-CCCGGCAATTCAAGAAG-3′ (SEQ. ID NO> 5). Total RNA obtained from primary fetal rat calvarial osteoblasts, was used for the primer extension. The results were shown in Figure 6. The major extension product was 68bp and was used to estimate the major transportation start site (+1, Figure 5). These results were confirmed by Rnase protection assays.

(c) Identification of BMP-2 Promoter and Enhancer

Activity Using Luciferase (LUC) Reporter Gene Constructs.

The BMP-2-LUC constructs (Figure 7) were designed to contain variable 5′ boundaries from BMP-2 5'-flanking sequences spanning the transcription start site (+1).

Each construct contained the 3' boundary at +114 9 in exon 1 (Figure 6). These constructs were individually transfected into primary cultures of fetal rat calvarial osteoblasts, ROS 17/2.8 osteosarcoma cells, HeLa cells, and CV-1 cells by the calcium-phosphate precipitation technique and the promoter activity for each of these constructs was assayed 24 hrs following transfection by measuring the luciferase enzyme activity for each individual cell lysate. The LUC (luciferase enzyme assay) technique is described below under (f). Plasmid psvβGal was co-transfected with each plasmid construct to normalize for the transfection efficiency in each sample. The experiments were repeated at least five times in independent fetal rat calvarial cultures, with each assay done in triplicate. The mean values from a representative experiment are shown in Figure 8.

(d) Isolation of Primary Fetal Rat Calvarial Osteoblasts for Functional Studies of BMP-2 Gene Promoter.

The cells used for transient transfection studies were isolated from 19 day-old fetal rat calvariae by sequential digestion with trypsin and collagenase, as described by Bellow *et al.*, (1986) and Harris *et al.*, (1994). In brief, the calvarial bone were surgically removed and cleaned by washing in α minimal essential media (αMEM) containing 10% V/V fetal calf serum (FCS) and antibiotics. The bones were minced with scissors and was transferred to 35 mm tissue culture dish containing 5 ml of sterile bacterial collagenase (0.1%) and trypsin (0.05%). This was then incubated at 37°C for 20 min. The cells released at this time were collected and immediately mixed with an equal volume of FCS to inactivate trypsin. This procedure was repeated 6 times to release cells at 20 min intervals. Cells released from 3rd, 4th, 5th and 6th digestion (enriched for osteoblasts) were combined and the cells were collected by centrifugation at 400 g for 5 min. The cells were then plated in αMEM containing 10% FCS and antibiotics and were grown to confluency (2-3 days). At this stage the cells were plated for transfection in 60 mm tissue culture dishes at a cell density of 5 x 10^5 cells per dish. These primary osteoblst cultures are capable f mineralized bone in prolonged cultures (Bellows *et* al, 1986; Harris *et* al, 1994). HeLa, ROS 17/2.8, and CV-1 cells were purchased from the ATCC.

(e) Transient Transfection Assay.

For transient transfection assay, the primary osteoblast cells were plated at the above mentioned cell density 18-24 hrs prior to transfection. The transfection was carried out using a modified calcium-phosphate precipitation method (Graham & van der Eb 1973;

Frost & Williams 1978). The cells were incubated for 4 hrs. at 37°C with 500µl of a calcium phosphate precipitate of plasmid DNA containing 10µg of reporter plasmid construct and 1µg of pSVβGal (for normalization of transfection efficiency) in 0.15M $CaCl_2$ and Hepes buffered saline (21mM Hepes, 13.5mM NaCl, 5mM KCl, 0.7mM Na_2HPO_4, 5.5mM dextrose, pH 7.05-7.1). After the 4 hr. incubation period of cells with precipitate, the cells were subjected to a 2 min treatment of 15% glycerol in αMEM, followed by addition of fresh αMEM containing insulin, transferrin and selenium (ITS) (Upstate Biotechnology Lake Placid, NY). The cells were harvested 24 hrs post transfection.

(f) Luciferase and β-galactosidase Assay.

Cells lysates were prepared and luciferase enzyme assay was carried out using assay protocols and the assay kit from Promega (Madison, WI). Routinely 20µl of cell lysate was mixed with 100µl of luciferase assay reagent (270µM coenzyme A, 470µM luciferin and 530µM ATP) and the luciferase activity was measured for 10 sec in a TURNER TD-20e luminometer. The values were normalized with respect to the β-galactosidase enzyme activity, obtained for each experimental sample

The β-galactosidase enzyme activity was measured in the cell lysate using a 96 well microtiter plate according to Rouet *et al.* (1992). 10-20µl cell lysate was added to 90-80µl β-galactosidase reaction buffer containing 88mM phosphate buffer, PH 7.3, 11mM KCL, 1mM $MgCl_2$, 55mM β mercaptoethanol, 4.4mM chlorophenol red β-D-galactopyranoside (Boehringer-Mannheim Corp., Indianapolis, IN). The reaction mixture was incubated at 37°C for 30-60 min, depending on transfection efficiency, and the samples were read with an ELISA plate reader at 600nm.

(g) Plasmid Construction

The luciferase basic plasmid (pGL basic) was the vector used for all constructs (purchased from Promega, Madison, WI). Different lengths of DNA fragments from the BmP-2 5′-flanking region were cloned at the multiple cloning sites of this plasmid, which is upstream of the firefly luciferase cDNA. The BMP-2 DNA fragments were isolated either by using available restriction enzyme sites (constructs -196/+114, -876/+114, -1995/+114, -2483/+114, and -2736/+114) or by polymerase chain reaction using specific oligonucleotide primers (constructs -23/+114, -123/+114 and +29/+114.

The minimal promoter activity for the BMP-2 gene was identified in the shortest construct containing 23bp upstream of the transcription start site (-23/+114). No luciferase activity was noted in the construct and did not include the transcription start site (+29/+114). Two other constructs containing increasing lengths of 5′ sequences up to -196bp showed reproducible decreases in promoter activity in fetal rat calvarial osteoblasts and HeLa cells (Figure 8). The -876/+114 construct showed a 5-fold increase in activity in HeLa cells. The -1995/+114, -2483/+114 and -2736/+114 constructs showed decreased promoter activity when compared to the -876/+114 construct only in HeLa cells (Figure 8).

In the primary fetal rat calvarial osteoblasts, the 2.6kb construct (-2483/+114) demonstrated a 2-3-fold increase in luciferase activity over that of the -1995/+114 construct (Figure 8). These results suggest that one or more positive response regions are present between -196 and -1995 and that the DNA sequence between -1995 and -2483bp was other positive regulatory elements that could modulate BMP-2 transcription. The largest 2.9kb construct (-2836/+114) repeatedly demonstrated a 20-50% decrease in promoter activity compared to the -2483/+114 construct, in these primary fetal rat calvarial osteoblasts (Figure 8).

In ROS 17/2.8 osteosarcoma cells, the BMP-2 promoter activity was consistently higher than either the primary fetal rat calvarial osteoblasts or HeLa cells (Figure 8). All of the deletion constructs showed similar promoter activity in ROS 17/2.8 osteosrcoma cells. The transformed state in ROS 17/2.8 cells may be responsible for the marked expression of the BMP-2 gene. ROS 17/2.8 cells represent a well differentiated osteosrcoma and they produce high levels of BMP-2 mRNA. They form tumors in nude mice with bone-like material in the tumor (Majeska *et al*, 1978; Majeska *et al*, 1980).

(h) Specificity of the BMP-2 Promoter.

To analyze the activity of the BMP-2 promoter in cell types not expressing BMP-2 mRNA, BMP-2 promoter constructs were transfected into CV-1 cells (monkey kidney cells). The BMP-2 promoter activity was found to be very low for all constructs. This suggests that this region of the BMP-2 promoter is functional only in cells such as primary fetal rat calvarial osteoblasts, HeLa and ROS 17/2.8 that express endogenous BMP-2 mRNA (Anderson & Coulter 1968). CV-1 cells do not express BMP-2 mRNA. The

BMP-2 promoter is likely active in other cell types that express BMP-2, such as prostate cells and chondrocytes, although regulation of transcription may be different in these cells.

Example 3: USE OF PLASMID CONSTRUCTS CONTAINING BMP PROMOTERS WITH REPORTER GENES TO IDENTIFY OSTEOGENIC AGENTS

Plasmid constructs containing BMP promoters with reporter genes have been transfected into osteoblastic cells. The cells which have been utilized include primary cultures of fetal rat calvarial osteoblasts, cell lines obtained as gifts or commercially (MC3T3-E12 cells, MG-63 cells, U2OS cells, UMR106 cells, ROS 17/2.8 cells, Sa)S2 cells, and the like as provided in the catalog from the ATCC) and bone and cartilage cell lines established from transgenic mice. The bone cells are transfected transiently or stably with the plasmid constructs, exposed to the chemical compound, agent or factor to be tested for 48 hours, and then luciferase or CAT activity is measure in the cell lysates.

Regulation of expression of the growth factor is assessed by culturing bone cells in αMEM medium with 10% fetal calf serum and 1% penicillin/streptomycin and 1% glutamine. The cells are placed in microtiter plates at a cell density of $5x10^3$ cells /100μl/well. The cells are allowed to adhere and then incubated at 37°C at 5% CO_2 for 24 hours and then the media is removed and replaced with 50μl αMEM and 4% fetal calf serum, 50μl aliquots containing the compound or factor to be tested in 0.1% BSA solution is added to each well. The final volume is 100μl and the final serum concentration is 2% fetal calf serum. Recombinant rat BMP-2 expressed in Chinese hamster ovarian cells is used as a positive control.

The treated cells are incubated at 37°C at 5% CO_2 for 48 hours. The media is then removed and the cells are rinsed 3 times with phosphate buffered saline (PBS). Excess PBS is removed from the wells and 100μl of cell culture lysing reagent (Promega #E153A) is added to each well. After 10 minutes, 10μl of the cell lysate is added to a 96-well white luminometric plate (Dynatech Labs #07100) containing 100μl luciferase assay buffer with substrate (Promega #E152A). The luciferase activity is read using a Dynatech ML2250 automated 96-well luminometer. The data is expressed as either picograms of luciferase activity per well or picograms of luciferase per μg protein.

Example 4: DEMONSTRATION THAT BONE CELLS TRANSFECTED WITH BMP PROMOTERS CAN BE USED TO SCREEN FOR OSTEOGENIC AGENTS

To demonstrate that the present invention is useful in evaluating potential osteogenic agents, a random array of chemical compounds from a chemical library obtained commercially was screened. It was found that approximately 1 in 100 such compounds screened produces a positive response in the present assay system compared with the positive control, recombinant BMP-2, which is known to enhance BMP-2 transcription. Compounds identified from the random library were subjected to detailed dose-response curves, to demonstrate that they enhance BMP messenger RNA expression, and that they enhance other biological effects *in vitro*, such as expression of structural proteins including osteocalcin, osteopontin and alkaline phosphase, and enhance bone nodule formation in prolonged primary cultures of calvarial rodent osteoblasts.

Compounds identified in this way can be tested for their capacity to stimulate bone formation *in vitro* in mice. To demonstrate this, the compound can be injected locally into subcutaneous tissue over the calvarium of normal mice and then the bone changes are followed histologically. It has been found that certain compounds identified by the present invention stimulate the formation of new bone in this *in vivo* assay system.

The effects of compounds are tested in ICR Swiss mice, aged 4-6 weeks and weighing 13-26g. The compound at 20mg/kg or vehicle along (100μl of 5% DMSO and phosphate-buffered 0.9% saline) are injected three times daily for 7 days. The injections are given into the subcutaneous tissues overlying the right side of the calvaria of five mice in each treatment group in each experiment.

Mice are killed by either inhalation on day 14, *i.e.* 7 days after the last injection of compound. After fixation in 10% phosphate-buffered formalin, the calvariae are examined. The occipital bone is removed by cutting immediately behind and parallel to the lambdoid suture, and the frontal bone is removed by cutting anterior to the coronal suture using a scalpel blade. The bones are then bisected through the coronal plane and the 3- to 4mm strips of bone are decalcified in 14% EDTA, dehydrated in graded alcohols, and embedded in paraffin. Four 3μm thick nonconsecutive step sections are cut from each specimen and stained using hematoxylin and eosin.

Two representative sections from the posterior calvarial strips are used. Histological measurements are carried out using a digitizing tablet and the Osteomeasure

image analysis system (Osteometrics Inc., Atlanta, GA) on the injected and noninjected sides of the calvariae in a standard length of bone between the sagittal suture and the muscle insertion of the lateral border of each bone. Measurements consist of (1) Total bone area (*i.e.*, bone and marrow between inner and outer periosteal surfaces); (2) Area of new woven bone formed on the outer calvarial surface; (3) The extent of osteoblast lined surface on the outer calvarial surface; (4) The area of the outer periosteum; and (5) The length of calvarial surface. From these measurements, the mean width of new bone and periosteum and the percentage of surface lined by osteoblasts on the outer calvarial surface, can be determined.

By reference to the above disclosure and examples, it is seen that the present invention provides a new cell-based assay for identifying and evaluating compounds which stimulate the growth of bone. Also provided in accordance with the present invention are promoter regions of bone morphogenetic protein genes, and a system for identifying osteogenic agents utilizing such promoters operatively linked to reporter genes in expression vectors.

The present invention provides the means to specifically identify osteogenic agents which stimulate bone cells to produce bone growth factors in the bone morphogenetic protein family. These osteogenic agents are shown to be useful to increase the activity of the promoters of genes of members of the BMP family and other bone growth factors normally produced by bone cells.

Example 5: RESEQUENCING OF THE BMP-2 5′ FLANKING REGION

The BMP-2 5′ flanking region described in Example 2 was resequenced. The nucleotide sequence of the 5′ flanking region of the mouse BMP-2 gene is provided in Figure 11. The sequence information in Figure 11 corrects sequencing errors that are present in Figures 5 and 9. The nucleotide sequence of Figure 11 replaces bases -2736 to +119 provided in Figure 5 and bases 1 to 2855 provided in Figure 9. The non-nucleotide sequence information provided in Figure 5 is applicable to the corresponding bases in Figure 11 where such bases are present.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application are [is] specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent to those of ordinary skill in the art in light of the teaching of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2310 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 768..1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TGGCCCTCGA CCAGGTTCAT TGCAGCTTTC TAGAGGTCCC CAGAAGCAGC TGCTGGCGAG    300

CCCGCTTCTG CAGGAACCAA TGGTGAGCTC GAGTGCAGGC CGAAAGCTGT TCTCGGGTTT    360

GTAGACGCTT GGGATCGCGC TTGGGGTCTC CTTTCGTGCC GGGTAGGAGT TGTAAAGCCT    420

TTGCAACTCT GAGATCGTAA AAAAAATGTG ATGCGCTCTT TCTTTGGCGA CGCCTGTTTT    480

GGAATCTGTC CGGAGTTAGA AGCTCAGACG TCCACCCCCC ACCCCCCGCC CACCCCCTCT    540

GCCTTGAATG GCACCGCCGA CCGGTTTCTG AAGGATCTGC TTGGCTGGAG CGGACGCTGA    600

GGTTGGCAGA CACGGTGTGG ATTTTAGGAG CCATTCCGTA GTGCCATTCG GAGCGACGCA    660

CTGCCGCAGC TTCTCTGAGC CTTTCCAGCA AGTTTGTTCA AGATTGGCTC CCAAGAATCA    720

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 20                  25                  30                  35

GCC GAG ATT CAG GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT      920
Ala Glu Ile Gln Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His
                 40                  45                  50

GAG CTC CTG CGG GAC TTC GAG GCG ACA CTT CTA CAG ATG TTT GGG CTG      968
Glu Leu Leu Arg Asp Phe Glu Ala Thr Leu Leu Gln Met Phe Gly Leu
             55                  60                  65

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         70                  75                  80

AGG GAT CTT TAC CGG CTC CAG TCT GGG GAG GAG GAG GAG GAA GAG CAG     1064
Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu Glu Glu Glu Glu Glu Gln
     85                  90                  95
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165 170 175

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Asn Ile Tyr Glu Val Met Lys Pro Pro Ala Glu Met Val Pro Gly His
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Leu Ile Thr Arg Leu Leu Asp Thr Arg Leu Val His His Asn Val Thr
200 205 210

CGG TGG GAA ACT TTC GAT GTG AGC CCT GCA GTC CTT CGC TGG ACC CGG 1448
Arg Trp Glu Thr Phe Asp Val Ser Pro Ala Val Leu Arg Trp Thr Arg
215 220 225

GAA AAG CAA CCC AAT TAT GGG CTG GCC ATT GAG GTG ACT CAC CTC CAC 1496
Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu Val Thr His Leu His
230 235 240

CAG ACA CGG ACC CAC CAG GGC CAG CAT GTC AGA ATC AGC CGA TCG TTA 1544
Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser Arg Ser Leu
245 250 255

CCT CAA GGG AGT GGA GAT TGG GCC CAA CTC CGC CCC CTC CTG GTC ACT 1592
Pro Gln Gly Ser Gly Asp Trp Ala Gln Leu Arg Pro Leu Leu Val Thr
260 265 270 275

TTT GGC CAT GAT GGC CGG GGC CAT ACC TTG ACC CGC AGG AGG GCC AAA 1640
Phe Gly His Asp Gly Arg Gly His Thr Leu Thr Arg Arg Arg Ala Lys
280 285 290

CGT AGT CCC AAG CAT CAC CCA CAG CGG TCC AGG AAG AAG AAT AAG AAC 1688
Arg Ser Pro Lys His His Pro Gln Arg Ser Arg Lys Lys Asn Lys Asn
295 300 305

TGC CGT CGC CAT TCA CTA TAC GTG GAC TTC AGT GAC GTG GGC TGG AAT 1736
Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn
310 315 320

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Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly
    325                 330                 335

GAC TGT CCC TTT CCA CTG GCT GAT CAC CTC AAC TCA ACC AAC CAT GCC      1832
Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala
340                 345                 350                 355

ATT GTG CAG ACC CTA GTC AAC TCT GTT AAT TCT AGT ATC CCT AAG GCC      1880
Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys Ala
                360                 365                 370

TGT TGT GTC CCC ACT GAA CTG AGT GCC ATT TCC ATG TTG TAC CTG GAT      1928
Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp
            375                 380                 385

GAG TAT GAC AAG GTG GTG TTG AAA AAT TAT CAG GAG ATG GTG GTA GAG      1976
Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val Glu
        390                 395                 400

GGG TGT GGA TGC CGC TGAGATCAGA CAGTCCGGAG GGCGGACACA CACACACACA      2031
Gly Cys Gly Cys Arg
    405

CACACACACA CACACACACA CACACACACA CGTTCCCATT CAACCACCTA CACATACCAC    2091

ACAAACTGCT TCCCTATAGC TGGACTTTTA TCTTAAAAAA AAAAAAAAGA AAGAAAGAAA    2151

GAAAGAAAGA AAAAAAATGA AAGACAGAAA AGAAAAAAAA AACCCTAAAC AACTCACCTT    2211

GACCTTATTT ATGACTTTAC GTGCAAATGT TTTGACCATA TTGATCATAT TTTGACAAAT    2271

ATATTTATAA AACTACATAT TAAAAGAAAA TAAAATGAG                           2310
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 408 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Ile Pro Gly Asn Arg Met Leu Met Val Val Leu Leu Cys Gln Val
 1               5                  10                  15

Leu Leu Gly Gly Ala Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys
            20                  25                  30

Lys Lys Val Ala Glu Ile Gln Gly His Ala Gly Gly Arg Arg Ser Gly
        35                  40                  45

Gln Ser His Glu Leu Leu Arg Asp Phe Glu Ala Thr Leu Leu Gln Met
    50                  55                  60
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Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys Ser Ala Val Ile Pro
65 70 75 80

Asp Tyr Met Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu Glu Glu Glu
85 90 95

Glu Glu Gln Ser Gln Gly Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala
100 105 110

Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu
115 120 125

Asn Ile Pro Gly Thr Ser Glu Ser Ser Ala Phe Arg Phe Leu Phe Asn
130 135 140

Leu Ser Ser Ile Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg
145 150 155 160

Leu Phe Arg Glu Gln Val Asp Gln Gly Pro Asp Trp Glu Gln Gly Phe
165 170 175

His Arg Ile Asn Ile Tyr Glu Val Met Lys Pro Pro Ala Glu Met Val
180 185 190

Pro Gly His Leu Ile Thr Arg Leu Leu Asp Thr Arg Leu Val His His
195 200 205

Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro Ala Val Leu Arg
210 215 220

Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu Val Thr
225 230 235 240

His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser
245 250 255

Arg Ser Leu Pro Gln Gly Ser Gly Asp Trp Ala Gln Leu Arg Pro Leu
260 265 270

Leu Val Thr Phe Gly His Asp Gly Arg Gly His Thr Leu Thr Arg Arg
275 280 285

Arg Ala Lys Arg Ser Pro Lys His His Pro Gln Arg Ser Arg Lys Lys
290 295 300

Asn Lys Asn Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val
305 310 315 320

Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr
325 330 335

Cys His Gly Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr
340 345 350

Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile
355 360 365

Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu
370 375 380

Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met
385 390 395 400

Val Val Glu Gly Cys Gly Cys Arg
405

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2688 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAATTCGCTA GGTAGACCAG GCTGGCCCAG AACACCTAGA GATCATCTGG CTGCCTCTGT 60

CTCTTGAGTT CTGGGGCTAA AGCATGCACC ACTCTACCTG GCTAGTTTGT ATCCATCTAA 120

ATTGGGGAAG AAAGAAGTAC AGCTGTCCCC AGAGATAACA GCTGGGTTTT CCCATCAAAC 180

ACCTAGAAAT CCATTTTAGA TTCTAAATAG GGTTTGTCAG GTAGCTTAAT TAGAACTTTC 240

AGACTGGGTT TCACAGACTG GTTGGGCCAA AGGTCACTTT ATTGTCTGGG TTTCAGCAAA 300

ATGAGACAAT AGCTGTTATT CAAACAACAT TTGGGTAAGG AAGAAAAATG AACAAACACC 360

ACTCTCCCTC CCCCCGCTCC GTGCCTCCAA ATCCATTAAA GGCAAAGCTG CACCCCTAAG 420

GACAACGAAT CGCTGCTGTT TGTGAGTTTA AATATTAAGG AACACATTGT GTTAATGATT 480

GGAGCAGCAG TGATTGATGT AGTGGCATTG GTGAGCACTG AATCCGTCCT TCAACCTGCT 540

ATGGGAGCAC AGAGCCTGAT GCCCCAGGAG TAATGTAATA GAGTAATGTA ATGTAATGGA 600

GTTTTAATTT TGTGTTGTTG TTTTAAATAA TTAATTGTAA TTTTGGCTGT GTTAGAAGCT 660

GTGGGTACGT TTCTCAGTCA TCTTTTCGGT CTGGTGTTAT TGCCATACCT TGATTAATCG 720

GAGATTAAAA GAGAAGGTGT ACTTAGAAAC GATTTCAAAT GAAAGAAGGT ATGTTTCCAA 780

TGTGACTTCA CTAAAGTGAC AGTGACGCAG GGAATCAATC GTCTTCTAAT AGAAAGGGCT 840

CATGGAGACC TGAGCTGAAT CTTTCTGTTC TGGATGAGAG AGGTGGTACC CATTGGAATG 900

AAAGGACTTA GTCAGGGGCA ATACAGTGTG CTCCAAGGCT GGGGATGGTC AGGATGTTGT 960

GCTCAGCCTC TAACACTCCT TCCAACCTGA CATTCCTTCT CACCCTTTGT CTCTGGCCAG 1020

TAGAATACAG GAACTCGTTC CTGTTTTTTT TTTTTTAAAT TCTGAAGGTG TGTAAGTACA 1080

AAGGTCAGAT GAGCGGCCCT AGGTCAAGAC TGCTTTGTGG TGACAAGGGA GTATAACACC 1140

CACCCCAGAA ACCAAGAACC GGAAATTGCT ATCTTCCAGC CCTTTGAGAG CTACCTGAAG 1200

CTCTGGGCTG CTGGCCTCAC CCCTTCCCTG CAGCTTTCCC TTTAGCAGAG GCTGTGATTT 1260

CCTTCAGCGC TTGGGCAAAT ACTCTTAGCC TGGCTCACCT TCCCCATCCT CGTTTGTAAA 1320

AACAAAGATG AAGCTGATAG TTCCTTCCCA GCTCCATCAG AGGCAGGGTG TGAAATTAGC 1380

TCCTGTTTGG GAAGGTTTAA AAGCCGGCCA CATTCCACCT CCCAGCTAGC ATGATTACCA 1440

ACTCTTGTTT CTTACTGTTG TTATGAAAGA CTCAATTCCT CATCTCCCTT TCCCTTCTTT 1500

TAAAAAGGGG CCAAAGGGCA CTTTGTTTTT TTCTCTACAT GGCCTAAAAG GCACTGTGTT 1560

ACCTTCCTGG AAGGTCCCAA ACAAACAAAC AAACAAACAA AATAACCATC TGGCAGTTAA 1620

GAAGGCTTCA GAGATATAAA TAGGATTTTC TAATTGTCTT ACAAGGCCTA GGCTGTTTGC 1680

CTGCCAAGTG CCTGCAAACT ACCTCTGTGC ACTTGAAATG TTAGACCTGG GGGATCGATG 1740

GAGGGCACCC AGTTTAAGGG GGGTTGGTGC AATTCTCAAA TGTCCACAAG AAACATCTCA 1800

CAAAAACTTT TTTGGGGGGA AAGTCACCTC CTAATAGTTG AAGAGGTATC TCCTTCGGGC 1860

ACACAGCCCT GCTCACAGCC TGTTTCAACG TTTGGGAATC CTTTAACAGT TTACGGAAGG 1920

CCACCCTTTA AACCAATCCA ACAGCTCCCT TCTCCATAAC CTGATTTTAG AGGTGTTTCA 1980

TTATCTCTAA TTACTCGGGG TAAATGGTGA TTACTCAGTG TTTTAATCAT CAGTTTGGGC 2040

AGCAGTTATT CTAAACTCAG GGAAGCCCAG ACTCCCATGG GTATTTTTGG AAGGTACAGA 2100

GACTAGTTGG TGCATGCTTT CTAGTACCTC TTGCATGTGG TCCCCAGGTG AGCCCCGGCT 2160

GCTTCCCGAG CTGGAGGCAT CGGTCCCAGC CAAGGTGGCA ACTGAGGGCT GGGGAGCTGT 2220

GCAATCTTCC GGACCCGGCC TTGCCAGGCG AGGCGAGGCC CCGTGGCTGG ATGGGAGGAT 2280

GTGGGCGGGG CTCCCCATCC CAGAAGGGGA GGCGATTAAG GGAGGAGGGA AGAAGGGAGG 2340

GGCCGCTGGG GGGAAAGACT GGGGAGGAAG GGAAGAAAGA GAGGGAGGGA AAAGAGAAGG 2400

AAGGAGTAGA TGTGAGAGGG TGGTGCTGAG GGTGGGAAGG CAAGAGCGCG AGGCCTGGCC 2460

CGGAAGCTAG GTGAGTTCGG CATCCGAGCT GAGAGACCCC AGCCTAAGAC GCCTGCGCTG 2520

CAACCCAGCC TGAGTATCTG GTCTCCGTCC CTGATGGGAT TCTCGTCTAA ACCGTCTTGG 2580

AGCCTGCAGC GATCCAGTCT CTGGCCCTCG ACCAGGTTCA TTGCAGCTTT CTAGAGGTCC 2640

CCAGAAGCAG CTGCTGGCGA GCCCGCTTCT GCAGGAACCA ATGGTGAG 2688

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2875 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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GAATTCATTT AAGCTGGATT CACTTCTAGG TCCCATGCGT TTACACTCAT TTCCACCACA     60
AGAGGGCAGC CATCTCTAAA AAAACAACAG TCGAGTGCTC TTCAGAGAAA TTGGGCCAAA    120
CTTGAGGAAA GTTCCTGGGA AAGGCTTTTT AGCAGCACCT CTCTGGGCTA CAAAAAAGAA    180
GCCAGCAGGC ACCACCAAGG TGGAGTAACT GTCCAGAGGC ATCCATTTTA CCTCAGAGAC    240
TTGATTACTA AGGATATCCT AAACGGCCAA ACTCTCTCTT CTGGTGTTCC AGAGGCCCAA    300
AGCTGCAAGG CATTGTTGAT GTCATCACCA AAGGTTTCAT TTTCATCTTT TCTTGGGGTT    360
GGTCCAACAG CTGTCAGCTT TCTCTTCCTC ATTAAAGGCA ACTTTCTCAT TTAAATCTCA    420
TATAGGTTCG GAGTTTCTTG CTTTGCTCCT TCCGCCTCCG CGATGACAGA AGCAATGGTT    480
AACTTCTCAA TTAAACTTGA TAGGGAAGGA AATGGCTTCA GAGGCGATCA GCCCTTTTGA    540
CTTACACACT TACACGTCTG AGTGGAGTGT TTTATTGCCG CCTTGTTTGG TGTCTCATGA    600
TTCAGAGTGA CAACTTCTGC AACACGTTTT AAAAAGGAAT ACAGTAGCTG ATCGCAAATT    660
GCTGGATCTA TCCCTTCCTC TCCTTTAATT TCCCTTGTAG ACAGCCTTCC TTCAAAAATA    720
CCTTATTTGA CCTCTACAGC TCTAGAAACA GCCAGGGCCT AATTTCCCTC TGTGGGTTGC    780
TAATCCGATT TAGGTGAACG AACCTAGAGT TATTTTAGCT AAAAGACTGA AAAGCTAGCA    840
CACGTGGGTA AAAAAATCAT TAAAGCCCCT GCTTCTGGTC TTTCTCGGTC TTTGCTTTGC    900
AAACTGGAAA GATCTGGTTC ACAACGTAAC GTTATCACTC TGGTCTTCTA CAGGAATGCT    960
CAGCCCATAG TTTTGGGGGT CCTGTGGGTA GCCAGTGGTG GTACTATAAG GCTCCTGAAT   1020
GTAGGGAGAA ATGGAAAGAT TCAAAAAAGA ATCCTGGCTC AGCAGCTTGG GGACATTTCC   1080
AGCTGAGGAA GAAAACTGGC TTGGCCACAG CCAGAGCCTT CTGCTGGAGA CCCAGTGGAG   1140
AGAGAGGACC AGGCAGAAAA TTCAAAGGTC TCAAACCGGA ATTGTCTTGT TACCTGACTC   1200
TGGAGTAGGT GGGTGTGGAA GGGAAGATAA ATATCACAAG TATCGAAGTG ATCGCTTCTA   1260
TAAAGAGAAT TTCTATTAAC TCTCATTGTC CCTCACATGG ACACACACAC ACACACACAC   1320
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ACACACACAC ACACATCACT AGAAGGGATG TCACTTTACA AGTGTGTATC TATGTTCAGA 1380
AACCTGTACC CGTATTTTTA TAATTTACAT AAATAAATAC ATATAAAATA TATGCATCTT 1440
TTTATTAGAT TCATTTATTT GAATATAAAT GTATGAATAT TTATAAAATG TAATAATGCA 1500
CTCAGATGTG TATCGGCTAT TTCTCGACAT TTTCTTCTCA CCATTCAAAA CAGAAGCGTT 1560
TGCTCACATT TTTGCCAAAA TGTCTAATAA CTTGTAAGTT CTGTTCTTCT TTTTAATGTG 1620
CTCTTACCTA AAAACTTCAA ACTCAAGTTG ATATTGGCCC AATGAGGGAA CTCAGAGGCC 1680
AGTGGACTCT GGATTTGCCC TAGTCTCCCG CAGCTGTGGG CGCGGATCCA GGTCCCGGGG 1740
GTCGGCTTCA CACTCATCCG GGACGCGACC CCTTAGCGGC CGCGCGCTCG CCCCGCCCCG 1800
CTCCACCGCG GCCCCGTACG CGCCGTCCAC ACCCCTGCGC GCCCGTCCCG CCCGCCCGGG 1860
GGATCCCGGC CGTGCTGCCT CCGAGGGGGA GGTGTTCGCC ACGGCCGGGA GGGAGCCGGC 1920
AGGCGGCGTC TCCTTTAAAA GCCGCGAGCG CGCGCCAGCG CGGCTCGTCG CCGCCGGAGT 1980
CCTCGCCCTG CCGCGCAGAG CCCTGCTCGC ACTGCGCCCG CCGCGTGCGC TTCCCACAGC 2040
CCGCCCGGGA TTGGCAGCCC CGGACGTAGC CTCCCCAGGC GACACCAGGC ACCGGGACGC 2100
CCTCCCGGCG AAAGACGCGA GGGTCACCCG CGGCTTCGAG GGACTGGCAC GACACGGGTT 2160
GGAACTCCAG ACTGTGCGCG CCTGGCGCTG TGGCCTCGGC TGTCCGGGAG AAGCTAGAGT 2220
CGCGGACCGA CGCTAAGAAC CGGGAGTCCG GAGCACAGTC TTACCCTCAA TGCGGGGCCA 2280
CTCTGACCCA GGAGTGAGCG CCCAAGGCGA TCGGGCGGAA GAGTGAGTGG ACCCCAGGCT 2340
GCCACAAAAG ACACTTGGCC CGAGGGCTCG GAGCGCGAGG TCACCCGGTT TGGCAACCCG 2400
AGACGCGCGG CTGGACTGTC TCGAGAATGA GCCCCAGGAC GCCGGGGCGC CGCAGCCGTG 2460
CGGGCTCTGC TGGCGAGCGC TGATGGGGGT GCGCCAGAGT CAGGCTGAGG GAGTGCAGAG 2520
TGCGGCCCGC CCGCCACCCA AGATCTTCGC TGCGCCCTTG CCCGGACACG GCATCGCCCA 2580
CGATGGCTGC CCCGAGCCAT GGGTCGCGGC CCACGTAACG CAGAACGTCC GTCCTCCGCC 2640
CGGCGAGTCC CGGAGCCAGC CCCGCGCCCC GCCAGCGCTG GTCCCTGAGG CCGACGACAG 2700
CAGCAGCCTT GCCTCAGCCT TCCCTTCCGT CCCGGCCCCG CACTCCTCCC CCTGCTCGAG 2760
GCTGTGTGTC AGCACTTGGC TGGAGACTTC TTGAACTTGC CGGGAGAGTG ACTTGGGCTC 2820
CCCACTTCGC GCCGGTGTCC TCGCCCGGCG GATCCAGTCT TGCCGCCTCC AGCCC 2875

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCCGGCAAGT TCAAGAAG 18

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15144 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GAATTCATTT AAGCTGGATT CACTTCTAGG TCCCATGCGT TTACACTCAT TTCCACCACA 60

AGAGGGCAGC CATCTCTAAA AAAACAACAG TCGAGTGCTC TTCAGAGAAA TTGGGCCAAA 120

CTTGAGGAAA GTTCCTGGGA AAGGCTTTTT AGCAGCACCT CTCTGGGCTA CAAAAAAGAA 180

GCCAGCAGGC ACCACCAAGG TGGAGTAACT GTCCAGAGGC ATCCATTTTA CCTCAGAGAC 240

TTGATTACTA AGGATATCCT AAACGGCCAA ACTCTCTCTT CTGGTGTTCC AGAGGCCCAA 300

AGCTGCAAGG CATTGTTGAT GTCATCACCA AAGGTTTCAT TTTCATCTTT TCTTGGGGTT 360

GGTCCAACAG CTGTCAGCTT TCTCTTCCTC ATTAAAGGCA ACTTTCTCAT TTAAATCTCA 420

TATAGGTTCG GAGTTTCTTG CTTTGCTCCT TCCGCCTCCG CGATGACAGA AGCAATGGTT 480

AACTTCTCAA TTAAACTTGA TAGGGAAGGA AATGGCTTCA GAGGCGATCA GCCCTTTTGA 540

CTTACACACT TACACGTCTG AGTGGAGTGT TTTATTGCCG CCTTGTTTGG TGTCTCATGA 600

TTCAGAGTGA CAACTTCTGC AACACGTTTT AAAAAGGAAT ACAGTAGCTG ATCGCAAATT 660

GCTGGATCTA TCCCTTCCTC TCCTTTAATT TCCCTTGTAG ACAGCCTTCC TTCAAAAATA 720

CCTTATTTGA CCTCTACAGC TCTAGAAACA GCCAGGGCCT AATTTCCCTC TGTGGGTTGC 780

TAATCCGATT TAGGTGAACG AACCTAGAGT TATTTTAGCT AAAAGACTGA AAAGCTAGCA 840

CACGTGGGTA AAAAAATCAT TAAAGCCCCT GCTTCTGGTC TTTCTCGGTC TTTGCTTTGC 900

AAACTGGAAA GATCTGGTTC ACAACGTAAC GTTATCACTC TGGTCTTCTA CAGGAATGCT 960

CAGCCCATAG TTTTGGGGGT CCTGTGGGTA GCCAGTGGTG GTACTATAAG GCTCCTGAAT 1020

GTAGGGAGAA ATGGAAAGAT TCAAAAAAGA ATCCTGGCTC AGCAGCTTGG GGACATTTCC 1080

AGCTGAGGAA GAAAACTGGC TTGGCCACAG CCAGAGCCTT CTGCTGGAGA CCCAGTGGAG 1140

AGAGAGGACC AGGCAGAAAA TTCAAAGGTC TCAAACCGGA ATTGTCTTGT TACCTGACTC 1200

TGGAGTAGGT GGGTGTGGAA GGGAAGATAA ATATCACAAG TATCGAAGTG ATCGCTTCTA 1260

TAAAGAGAAT TTCTATTAAC TCTCATTGTC CCTCACATGG ACACACACAC ACACACACAC 1320

ACACACACAC ACACATCACT AGAAGGGATG TCACTTTACA AGTGTGTATC TATGTTCAGA 1380

AACCTGTACC CGTATTTTTA TAATTTACAT AAATAAATAC ATATAAAATA TATGCATCTT 1440

TTTATTAGAT TCATTTATTT GAATATAAAT GTATGAATAT TTATAAAATG TAATAATGCA 1500

CTCAGATGTG TATCGGCTAT TTCTCGACAT TTTCTTCTCA CCATTCAAAA CAGAAGCGTT 1560

TGCTCACATT TTTGCCAAAA TGTCTAATAA CTTGTAAGTT CTGTTCTTCT TTTTAATGTG 1620

CTCTTACCTA AAAACTTCAA ACTCAAGTTG ATATTGGCCC AATGAGGGAA CTCAGAGGCC 1680

AGTGGACTCT GGATTTGCCC TAGTCTCCCG CAGCTGTGGG CGCGGATCCA GGTCCCGGGG 1740

GTCGGCTTCA CACTCATCCG GGACGCGACC CCTTAGCGGC CGCGCGCTCG CCCCGCCCCG 1800

CTCCACCGCG GCCCCGTACG CGCCGTCCAC ACCCCTGCGC GCCCGTCCCG CCCGCCCGGG 1860

GGATCCCGGC CGTGCTGCCT CCGAGGGGGA GGTGTTCGCC ACGGCCGGGA GGGAGCCGGC 1920

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CGGGCTCTGC TGGCGAGCGC TGATGGGGGT GCGCCAGAGT CAGGCTGAGG GAGTGCAGAG 2520

TGCGGCCCGC CCGCCACCCA AGATCTTCGC TGCGCCCTTG CCCGGACACG GCATCGCCCA 2580

CGATGGCTGC CCCGAGCCAT GGGTCGCGGC CCACGTAACG CAGAACGTCC GTCCTCCGCC 2640
CGGCGAGTCC CGGAGCCAGC CCCGCGCCCC GCCAGCGCTG GTCCCTGAGG CCGACGACAG 2700
CAGCAGCCTT GCCTCAGCCT TCCCTTCCGT CCCGGCCCCG CACTCCTCCC CCTGCTCGAG 2760
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CCCACTTCGC GCCGGTGTCC TCGCCCGGCG GATCCAGTCT TGCCGCCTCC AGCCCGATCA 2880
CCTCTCTTCC TCAGCCCGCT GGCCCACCCC AAGACACAGT TCCCTACAGG GAGAACACCC 2940
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GGACAGAGCT TTTTCCATGT GGAGACTCTC TCAATGGACG TGCCCCCTAG TGCTTCTTAG 3060
ACGGACTGCG GTCTCCTAAA GGTAGAGGAC ACGGGCCGGG GACCCGGGGT TGGCTGGCGG 3120
GTGACACCGC TTCCCGCCCA ACGCAGGGCG CCTGGGAGGA CTGGTGGAGT GGAGTGGACG 3180
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CCCCAGATCC CTCTGCCTTG CCCACTGGCC TCCTTCCTCC AGCCGGTTCC TCCTCCCCAA 3300
GTTTTCGATA CATTATAAGG GCTGTTTTGG GCTTTCAAAA AAAAAAATGC AGAAATCCAT 3360
TTAAGAGTAT GGCCAGTAGA TTTTACTAGT TCATTGCTGA CCAGTAAGTA CTCCAAGCCT 3420
TAGAGATCCT TGGCTATCCT TAAGAAGTAG GTCCATTTAG GAAGATACTA AAAGTTGGGG 3480
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CACTATCTAT TTAGTTAATT GCAGGAAGGT GCATGGATTT CTTGACTGCA CAGGAGTCTT 3600
GGGGAAGGGG GAACAGGGTT GCCTGTGGGT CAACCTTAAA TAGTTAGGGC GAGGCCACAA 3660
CTTGCAAGTG GCGTCATTAG CAGTAATCTT GAGTTTAGCG CTTACTGAAT CTACAAGTTT 3720
GATATGCTCA ACTACCAGGA AATTGTATAC AGCGCCTCTA AGGAAGTCAC TTGTGCATTT 3780
GTGTCTGTTA ATATGCACAT GAGGCTGCAC TGTATAAGTT TGTCAGGGAT GCAGTGTCCG 3840
ACCAACCTAT GGCTTCCCAG CTTCCTGACA CCCGCATTCC CAGCTAGTGT CACAAGAAAA 3900
GGGTACAGAC GGTCAAGCTC TTTTTAATTG GGAGTTAAGA CCAAGCCCCA AGTAAGAAGT 3960
CCGGCTGGGA CTTGGGGGTC CTCCATCGGC CAGCGAGCTC TATGGGAGCC GAGGCGCGGG 4020
GGCGGCGGAG GACTGGGCGG GGAACGTGGG TGACTCACGT CGGCCCTGTC CGCAGGTCGA 4080
CCATGGTGGC CGGGACCCGC TGTCTTCTAG TGTTGCTGCT TCCCCAGGTC CTCCTGGGCG 4140
GCGCGGCCGG CCTCATTCCA GAGCTGGGCC GCAAGAAGTT CGCCGCGGCA TCCAGCCGAC 4200
CCTTGTCCCG GCCTTCGGAA GACGTCCTCA GCGAATTTGA GTTGAGGCTG CTCAGCATGT 4260
TTGGCCTGAA GCAGAGACCC ACCCCCAGCA AGGACGTCGT GGTGCCCCCC TATATGCTAG 4320

ATCTGTACCG CAGGCACTCA GGCCAGCCAG GAGCGCCCGC CCCAGACCAC CGGCTGGAGA 4380

GGGCAGCCAG CCGCGCCAAC ACCGTGCGCA CGTTCCATCA CGAAGGTGAG CGGGCGGCGG 4440

GTGGCGGGGC GGGGACGGCG GGCGGGCGGA GACTAGGCGG GCAGCCCGGG CCTCCACTAG 4500

CACAGTAGAA GGCCTTTCGG CTTCTGTACG GTCCCCTCTG TGGCCCCAGC CAGGGATTCC 4560

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CACAATTCGT CATATTGTTT CCACAATGAC CTTCGATTCT TTGCTTAAAT CTTAAATCTC 4980

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TGTTGTTTAT TTCAGCTGAA ATTTCCTGGA TTAGAAAGGC TGTAGAGGCC TATTGAAGTC 5160

TCTTGCACCG ATGTTCTGAA AGCAGTTAGT AAAAAATCAT GACCTAGCTC AATTCTGTGT 5220

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GTTCTAGAAG AATTCAGATT TAAAGTTTTG CTTTGCCTTG CTGAGGGGAT AAATTTTAAG 5340

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GGGCTTAGCT AATCTCTCCT GGTTAATATT CATTGGAAAG TTTTTATAGA TCAAAACAAA 5700

CAAACCTACT ATCCAGCACA GGTGTTTTTC CCACTGCCTC TGGAGATATA GCAAGAAAAC 5760

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AAAAATGAAG GAGGTATTTT ATCTTAACTA AGCTAAAAGA ATCGCTTAAG TCAATTGAAA 5880

CTCAAAAATC CAATTGAATG AAAGGTTCGT CAATAAAAAT CTACATTTTT CTTACTCTTC 5940

CTTTGGAAAT AGCTTGATAA AAACACAGAC AAAACAAAGT CTGTGTGCTT ATTTGAAAAC 6000

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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9299 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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TGGTGTCCCG CCGAGAGTTC CTCAGAGCAG GCGGGGACAA CTCCCAGACG GCTGGGGCTC 5400

CAGCTGCGGG CGCGGAGGTT GGCCTCGCTC GCAGGGGCTG GACCCAGCCG GGGTGGGAGG 5460

ATGGAGGAGG GGCGGGCGGG CTCTTCGGTG AGTGGGGCGG GGCCTCTGGG TCCACGTGAC 5520

TCCTAGGGGC TGGAAGAAAA ACAGAGCCTG TCTGCTCCAG AGTCTCATTA TATCAAATAT 5580

CATTTTAGGA GCCATTCCGT AGTGCCATTC GGAGCGACGC ACTGCCGCAG CTTCTCTGAG 5640

CCTTTCCAGC AAGTTTGTTC AAGATTGGCT CCCAAGAATC ATGGACTGTT ATTATGCCTT 5700

GTTTTCTGTC AGTGAGTAGA CACCTCTTCT TTCCCTTCTT GGGATTTCAC TCTGTCCTCC 5760

CATCCCTGAC CACTGTCTGT CCCTCCCGTC GGACTTCCAT TTCAGTGCCC CGCGCCCTAC 5820

TCTCAGGCAG CGCTATGGTT CTCTTTCTGG TCCCTGCAAG GCCAGACACT CGAAATGTAC 5880

GGGCTCCTTT TAAAGCGCTC CCACTGTTTT CTCTGATCCG CTGCGTTGCA AGAAAGAGGG 5940

AGCGCGAGGG ACCAAATAGA TGAAAGGTCC TCAGGTTGGG GCTGTCCCTT GAAGGGCTAA 6000

CCACTCCCTT ACCAGTCCCG ATATATCCAC TAGCCTGGGA AGGCCAGTTC CTTGCCTCAT 6060

AAAAAAAAAA AAAAAAACAA AAAACAAACA GTCGTTTGGG AACAAGACTC TTTAGTGAGC 6120

ATTTTCAACG CAGCGACCAC AATGAAATAA ATCACAAAGT CACTGGGGCA GCCCCTTGAC 6180

TCCTTTTCCC AGTCACTGGA CCTTGCTGCC CGGTCCAAGC CCTGCCGGCA CAGCTCTGTT 6240

CTCCCCTCCT CCTGTTCTTA ACCAGCTGGA AGTTGTGGAA ATTGGGCTGG AGGGCGGAGG 6300

AAGGGCGGGG GTGGGGGGGT GGAGAAGGTG GGGGGGGGGG AGGCTGAAGG TCCGAAGTGA 6360

AGAGCGATGG CATTTTAATT CTCCCTCCGC CTCCCCCCTT TACCTCCTCA ATGTTAACTG 6420

TTTATCCTTG AAGAAGCCAC GCTGAGATCA TGGCTCAGAT AGCCGTTGGG ACAGGATGGA 6480

GGCTATCTTA TTTGGGGTTA TTTGAGTGTA AACAAGTTAG ACCAAGTAAT TACAGGGCGA 6540

TTCTTACTTT CGGGCCGTGC ATGGCTGCAG CTGGTGTGTG TGTGTGTAGG GTGTGAGGGA 6600

GAAAACACAA ACTTGATCTT TCGGACCTGT TTTACATCTT GACCGTCGGT TGCTACCCCT 6660

ATATGCATAT GCAGAGACAT CTCTATTTCT CGCTATTGAT CGGTGTTTAT TTATTCTTTA 6720

ACCTTCCACC CCAACCCCCT CCCCAGAGAC ACCATGATTC CTGGTAACCG AATGCTGATG 6780

GTCGTTTTAT TATGCCAAGT CCTGCTAGGA GGCGCGAGCC ATGCTAGTTT GATACCTGAG 6840

ACCGGGAAGA AAAAAGTCGC CGAGATTCAG GGCCACGCGG GAGGACGCCG CTCAGGGCAG 6900

AGCCATGAGC TCCTGCGGGA CTTCGAGGCG ACACTTCTAC AGATGTTTGG GCTGCGCCGC 6960

CGTCCGCAGC CTAGCAAGAG CGCCGTCATT CCGGATTACA TGAGGGATCT TTACCGGCTC 7020

CAGTCTGGGG AGGAGGAGGA GGAAGAGCAG AGCCAGGGAA CCGGGCTTGA GTACCCGGAG 7080

CGTCCCGCCA GCCGAGCCAA CACTGTGAGG AGTTTCCATC ACGAAGGTCA GTTTCTGCTC 7140

TTAGTCCTGG CGGTGTAGGG TGGGGTAGAG CACCGGGGCA GAGGGTGGGG GGTGGGCAGC 7200

TGGCAGGGCA AGCTGAAGGG GTTGTGGAAG CCCCCGGGGA AGAAGAGTTC ATGTTACATC 7260

AAAGCTCCGA GTCCTGGAGA CTGTGGAACA GGGCCTCTTA CCTTCAACTT TCCAGAGCTG 7320

CCTCTGAGGG TACTTTCTGG AGACCAAGTA GTGGTGGTGA TGGGGGAGGG GGTTACTTTG 7380

GGAGAAGCGG ACTGACACCA CTCAGACTTC TGCTACCTCC CAGTGGGTGT TCTTTAGCTA 7440

TACCAAAGTC AGGGATTCTG CCCGTTTTGT TCCAAAGCAC CTACTGAATT TAATATTACA 7500

TCTGTGTGTT TGTCAGGTTT ATCAATAGGG GCCTTGTAAT ACGATCTGAA TGTTTCCTAG 7560

CGGATGTTTC TTTTCCAAAG TAAATCTGAG TTATTAATCC TCCAGCATCA TTACTGTGTT 7620

GGAATTTATT TTCCCTTCTG TAACATGATC AACAAGGCGT GCTCTGTGTT TCTAGGATCG 7680

CTGGGGAAAT GTTTGGTAAC ATACTCAAAA GTGGAGAGGG AGAGAGGGTG GCCCCTCTTT 7740

TTCTTTACAA CCACTTGTAA AGAAAACTGT ACACAAAGCC AAGAGGGGGC TTTAAAAGGG 7800

GAGTCCAAGG GTGGTGGAGT AAAAGAGTTG ACACATGGAA ATTATTAGGC ATATAAAGGA 7860

```
GGTTGGGAGA TACTTTCTGT CTTTGGTGTT TGACAAATGT GAGCTAAGTT TTGCTGGTTT   7920
GCTAGCTGCT CCACAACTCT GCTCCTTCAA ATTAAAAGGC ACAGTAATTT CCTCCCCTTA   7980
GGTTTCTACT ATATAAGCAG AATTCAACCA ATTCTGCTAT TTTTTGTTTT TGTTTCTTGT   8040
TTTTGTTTTG TTTGGTTTTT TTTTTTTTTT TTTTTTTTTT GTCTCAGAAA AGCTCATGGG   8100
CCTTTTCTTT TCCCCTTTCA ACTGTGCCTA GAACATCTGG AGAACATCCC AGGGACCAGT   8160
GAGAGCTCTG CTTTTCGTTT CCTCTTCAAC CTCAGCAGCA TCCCAGAAAA TGAGGTGATC   8220
TCCTCGGCAG AGCTCCGGCT CTTTCGGGAG CAGGTGGACC AGGGCCCTGA CTGGGAACAG   8280
GGCTTCCACC GTATAAACAT TTATGAGGTT ATGAAGCCCC CAGCAGAAAT GGTTCCTGGA   8340
CACCTCATCA CACGACTACT GGACACCAGA CTAGTCCATC ACAATGTGAC ACGGTGGGAA   8400
ACTTTCGATG TGAGCCCTGC AGTCCTTCGC TGGACCCGGG AAAAGCAACC CAATTATGGG   8460
CTGGCCATTG AGGTGACTCA CCTCCACCAG ACACGGACCC ACCAGGGCCA GCATGTCAGA   8520
ATCAGCCGAT CGTTACCTCA AGGGAGTGGA GATTGGGCCC AACTCCGCCC CCTCCTGGTC   8580
ACTTTTGGCC ATGATGGCCG GGGCCATACC TTGACCCGCA GGAGGGCCAA ACGTAGTCCC   8640
AAGCATCACC CACAGCGGTC CAGGAAGAAG AATAAGAACT GCCGTCGCCA TTCACTATAC   8700
GTGGACTTCA GTGACGTGGG CTGGAATGAT TGGATTGTGG CCCCACCCGG CTACCAGGCC   8760
TTCTACTGCC ATGGGGACTG TCCCTTTCCA CTGGCTGATC ACCTCAACTC AACCAACCAT   8820
GCCATTGTGC AGACCCTAGT CAACTCTGTT AATTCTAGTA TCCCTAAGGC CTGTTGTGTC   8880
CCCACTGAAC TGAGTGCCAT TTCCATGTTG TACCTGGATG AGTATGACAA GGTGGTGTTG   8940
AAAAATTATC AGGAGATGGT GGTAGAGGGG TGTGGATGCC GCTGAGATCA GACAGTCCGG   9000
AGGGCGGACA CACACACACA CACACACACA CACACACACA CACACACACA CACGTTCCCA   9060
TTCAACCACC TACACATACC ACACAAACTG CTTCCCTATA GCTGGACTTT TATCTTAAAA   9120
AAAAAAAAAA GAAAGAAAGA AAGAAAGAAA GAAAAAAAAT GAAAGACAGA AAAGAAAAAA   9180
AAAACCCTAA ACAACTCACC TTGACCTTAT TTATGACTTT ACGTGCAAAT GTTTTGACCA   9240
TATTGATCAT ATTTTGACAA ATATATTTAT AACTACATAT TAAAAGAAAA TAAAATGAG    9299
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGGATGCCGA ACTCACCTA 19

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTACAAACCC GAGAACAG 18

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCCGGCACGA AAGGAGAC 18

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAAGGCAAGA GCGCGAGG 18

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCCGGTCTCA GGTATCA 17

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CAGGCCGAAA GCTGTTC 17

Claims

1. A system for identifying osteogenic agents comprising a recombinant host cell modified to contain an expression sequence comprising a promoter derived from a gene encoding a bone morphogenic protein operatively linked to a reporter gene encoding an assayable product.

2. The system of claim 1 wherein said bone morphogenic protein is selected from the group consisting of the BMP-2 and BMP-4 proteins.

3. The system of claim 1 or 2 wherein said reporter gene comprises a gene encoding the production of an assayable product selected from the group consisting of firefly luciferase, chloramphenicol acetyl transferase, β-galactosidase, green fluorescent protein, human growth hormone, alkaline phosphatase and β-glucuronidase.

4. The system of claim 3 wherein said reporter gene comprises a gene encoding the production of firefly luciferase.

5. A method for identifying an osteogenic compound comprising the steps of:

culturing the cells of any of claim 1-4 under conditions which permit expression of said assayable product from said reporter gene;

contacting said cells with at least one candidate compound suspected of possessing osteogenic activity;

measuring the amount of assayable product produced in the presence of said candidate compound and comparing said amount to the amount of assayable product produced in the absence of said candidate compound; and

identifying, as an osteogenic compound, a candidate compound that enhances the amount of said assayable product when present.

6. An isolated nucleic acid molecule comprising a nucleotide sequence encoding the promoter region of a gene encoding bone morphogenetic protein selected from the group consisting of the BMP-2 and BMP-4 proteins.

7. The nucleic acid molecule of claim 6 which corresponds to a nucleotide sequence selected from the group consisting of positions -2372 to +316 of the BMP-4 gene depicted in Figure 1C (SEQ ID NO:3), a portion thereof which encodes a biologically active promoter, the BMP-2 sequence depicted in Figure 11, and a portion thereof which encodes a biologically active promoter.

8. A recombinant expression vector comprising the nucleotide sequence of claim 6 or 7.

9. The recombinant expression vector of claim 8 wherein said nucleotide sequence is operatively linked to a reporter gene encoding an assayable product.

10. The recombinant expression vector of claim 9 wherein said reporter gene comprises a gene encoding the production of an assayable product selected from the group consisting of firefly luciferase, chloramphenicol acetyl transferase, β-galactosidase, green fluorescent protein, human growth hormone, alkaline phosphatase or β-glucuronidase.

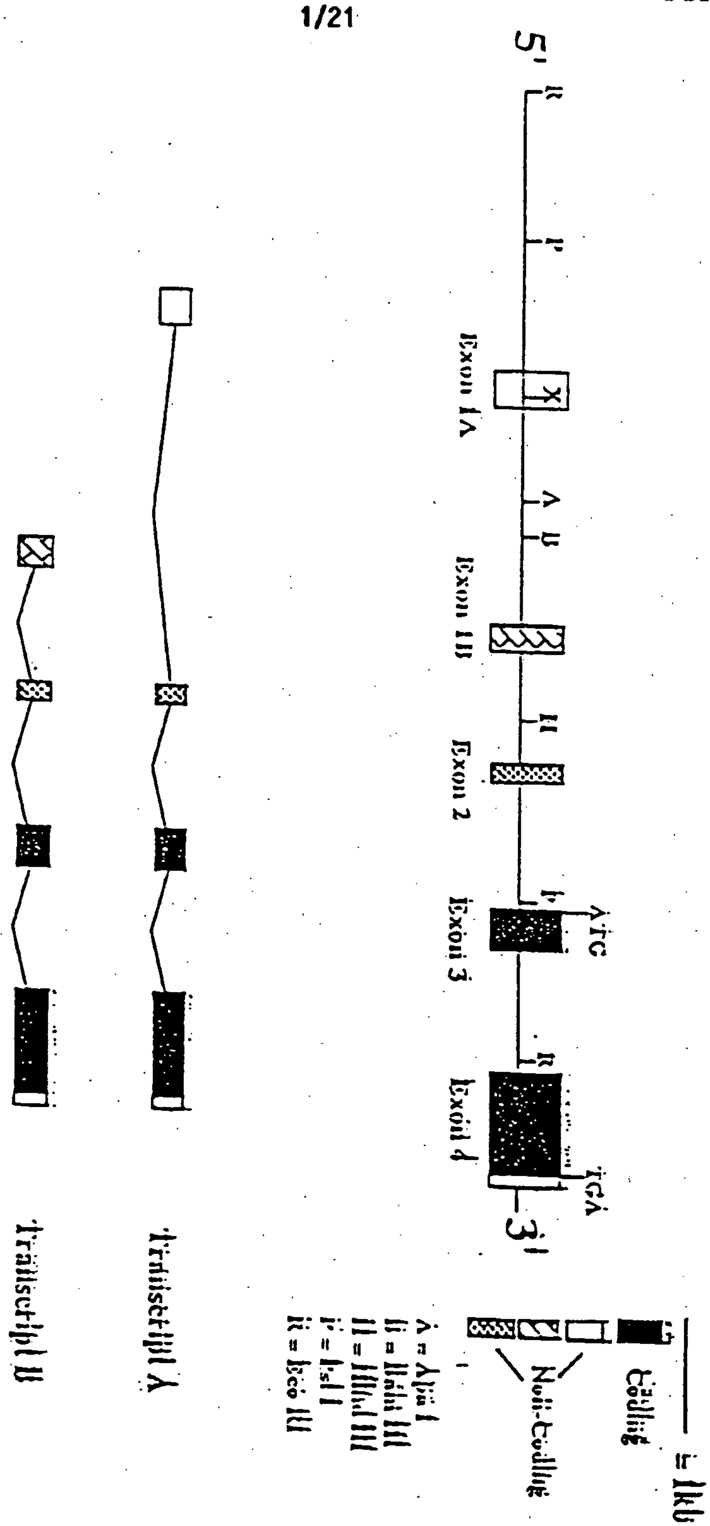


FIGURE 1A

```
                    5'FLANKING REGION            gggaggaaggGAAGAAAGAGAGGGAGGGAAAAGAGAAGGAAGGAGTAGA     39
                   5' -65--------------+82 3' Primer 1         EXON 1A
TGTGAGAGGGTGGTGCTGAGGGTGGGAAGGCAAGAGCGCGAGGCCTGCCCCGGAAGCTAGGTGAGTTCGGCATCCGAGCTGAGAGACCCCAGCCTAAGAC
GCCTGCGCTGCAACCCAGCCTGAGTATCTGGTCTCCGTCCCTGATGGGATTCTCGTCTAAACCGTCTTGGAGCCTGCAGCGATCCAGTCTCTGGCCCTCG     139
                                                                                                         239
ACCAGGTTCATTGCAGCTTTCTAGAGGTCCCCAGAAGCAGCTGCTGGCGAGCCCCGCTTCTGCAGGAACCAATGgtgagc...1822 bp..tcgagtGCA   2149
         +18                                                                INTRON I          +2 5' --
-------------- 3' Primer 2                                               EXON 1B
GGCCGAAAGCTGTTCTCGGGTTTGTAGACGCTTGGGATCGCGCTTGGGGTCTCCTTTCGTGCCGGGTAGGAGTTGTAAAGCCTTTGCAACTCTGAGATCG    2249
     3'                  5' Primer 81     3'                         5' Primer 82
      +13               +30                        +52              +69
TAAAAAAAATGTGATGCGCTCTTTCTTTGGCGACGCCTGTTTTGGAATCTGTCCGGAGTTAGAAGCTCAGACGTCCACCCCCCACCCCCCGCCCACCCCC    2349
TCTGCCTTGAATGGCACCGCCGACCGGTTTCTGAAGGATCTGCTTGGCTGGAGCGGACGCTGAGGTTGGCAGACACGgtgtgg...778 bp...atttc   3215
                                                                              INTRON II
                                                                  EXON II
agGAGCCATTCCGTAGTGCCATTCGGAGCGACGCACTGCCGCAGCTTCTCTGAGCCTTTCCAGCAAGTTTGTTCAAGATTGGCTCCCAAGAATCATGGAC   3315
                               INTRON III                         EXON III
TGTTATTATGCCTTGTTTTCTGTCAgtgagt..1025 bp..ccagagACACCATGATTCCTGGTAACCGAATGCTGATGGTCGTTTTATTATGCCAAGT   4429
                                                     M I P G N R M L N V V L L C Q V                16
                          3' ---------------- 5' Primer 3
CCTGCTAGGAGGCGCGAGCCATGCTAGTTTGATACCTGAGACCGGGAAGAAAAAAGTCGCCGAGATTCAGGGCCACGCGGGAGGACGCCGCTCAGGGCAG   4529
 L  L  G  G  A  S  H  A  S  L  I  P  E  T  G  K  K  K  V  A  E  I  Q  G  H  A  G  G  R  R  S  G  Q    49
AGCCATGAGCTCCTGCGGGACTTCGAGGCGACACTTCTACAGATGTTTGGGCTGCGCCGCCGTCCGCAGCCTAGCAAGAGCGCCGTCATTCCGGATTACA   4629
 S  H  E  L  L  R  D  F  E  A  T  L  L  Q  M  F  G  L  R  R  R  P  Q  P  S  K  S  A  V  I  P  D  Y  M    83
TGAGGGATCTTTACCGGCTCCAGTCTGGGGAGGAGGAGGAGGAAGAGCAGAGCCAGGGAACCGGGCTTGAGTACCCGGAGCGTCCCGCCAGCCGAGCCAA   4729
  R  D  L  Y  R  L  Q  S  G  E  E  E  E  E  E  Q  S  Q  G  T  G  L  E  Y  P  E  R  P  A  S  R  A  N    116
                                         INTRON IV                 EXON IV
CACTGTGAGGAGTTTCCATCACGAAGgtcagtttctg... 985 bp...tgtgcctagAACATCTGGAGAACATCCCAGGGACCAGTGAGAGCTCTGCT   5801
 T  V  R  S  F  H  H  E  E                                     N  L  E  N  I  P  G  T  S  E  S  S  A      138
TTTCGTTTCCTCTTCAACCTCAGCAGCATCCCAGAAAATGAGGTGATCTCCTCGGCAGAGCTCCGGCTCTTTCGGGAGCAGGTGGACCAGGGCCCTGACT   5901
 F  R  F  L  F  N  L  S  S  I  P  E  N  E  V  I  S  S  A  E  L  R  L  F  R  E  Q  V  D  Q  G  P  D  W    172
GGGAACAGGGCTTCCACCGTATAAACATTTATGAGGTTATGAAGCCCCCAGCAGAAATGGTTCCTGGACACCTCATCACACGACTACTGGACACCAGACT   6001
  E  Q  G  F  H  R  I  N  I  Y  E  V  M  K  P  P  A  E  M  V  P  G  H  L  I  T  R  L  L  D  T  R  L    205
AGTCCATCACAATGTGACACGGTGGGAAACTTTCGATGTGAGCCCTGCAGTCCTTCGCTGGACCCGGGAAAAGCAACCCAATTATGGGCTGGCCATTGAG   6101
 V  H  H  N  V  T  R  W  E  T  F  D  V  S  P  A  V  L  R  W  T  R  E  K  Q  P  N  Y  G  L  A  I  E    238
GTGACTCACCTCCACCAGACACGGACCCACCAGGGCCAGCATGTCAGAATCAGCCGATCGTTACCTCAAGGGAGTGGAGATTGGGCCCAACTCCGCCCCC   6201
 V  T  H  L  H  Q  T  R  T  H  Q  G  Q  H  V  R  I  S  R  S  L  P  Q  G  S  G  D  W  A  Q  L  R  P  L    272
TCCTGGTCACTTTTGGCCATGATGGCCGGGGCCATACCTTGACCCGCAGGAGGGCCAAACGTAGTCCCAAGCATCACCCACAGCGGTCCAGGAAGAAGAA   6301
  L  V  T  F  G  H  D  G  R  G  H  T  L  T  R  R  R  A  K  R  S  P  K  H  H  P  Q  R  S  R  K  K  N    305
TAAGAACTGCCGTCGCCATTCACTATACGTGGACTTCAGTGACGTGGGCTGGAATGATTGGATTGTGGCCCCACCCGGCTACCAGGCCTTCTACTGCCAT   6401
 K  N  C  R  R  H  S  L  Y  V  D  F  S  D  V  G  W  N  D  W  I  V  A  P  P  G  Y  Q  A  F  Y  C  H    338
GGGGACTGTCCCTTTCCACTGGCTGATCACCTCAACTCAACCAACCATGCCATTGTGCAGACCCTAGTCAACTCTGTTAATTCTAGTATCCCTAAGGCCT   6501
 G  D  C  P  F  P  L  A  D  H  L  N  S  T  N  H  A  I  V  Q  T  L  V  N  S  V  N  S  S  I  P  K  A  C    372
GTTGTGTCCCCACTGAACTGAGTGCCATTTCCATGTTGTACCTGGATGAGTATGACAAGGTGGTGTTGAAAAATTATCAGGAGATGGTGGTAGAGGGGTG   6601
  C  V  P  T  E  L  S  A  I  S  M  L  Y  L  D  E  Y  D  K  V  V  L  K  N  Y  Q  E  M  V  V  E  G  C    405
TGGATGCCGCTGAGATCAGACAGTCCGGAGGGCGGACACACACACACACACACACACACACACACACACACACACACACGTTCCCATTCAACCACCTA    6701
 G  C  R  END                                                                                           408
CACATACCACACAAACTGCTTCCCTATAGCTGGACTTTTATCTTAAAAAAAAAAAAAAGAAAGAAAGAAAGAAAGAAAGAAAAAAAATGAAAGACAGAAA  6801
AGAAAAAAAAAACCCTAAACAACTCACCTTGACCTTATTTATGACTTTACGTGCAAATGTTTTGACCATATTGATCATATTTTGACAAATATATTTATAA  6901
                       3'-FLANKING REGION
AACTACATATTAAAagaaaataaaatgag                                                                           6925
```

FIGURE 1B

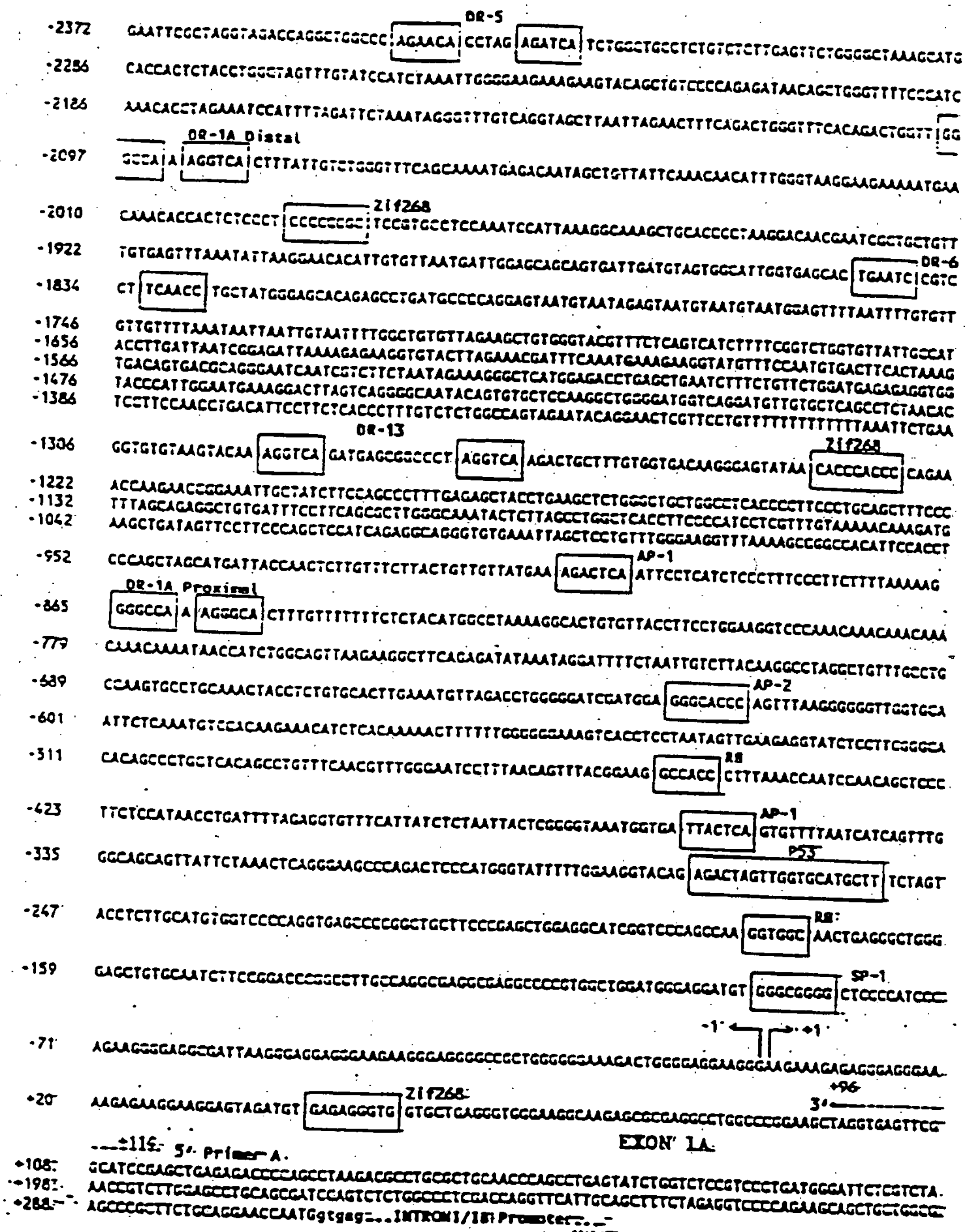

DR-5
DR-1A Distal
Zif268
DR-6
DR-13
Zif268
AP-1
DR-1A Proximal
AP-2
RB
AP-1
P53
RB
SP-1
-1
+1
Zif268
+96
3'
EXON 1A
+115 5' Primer A.
INTRON1/1B Promoter

FIGURE 1C

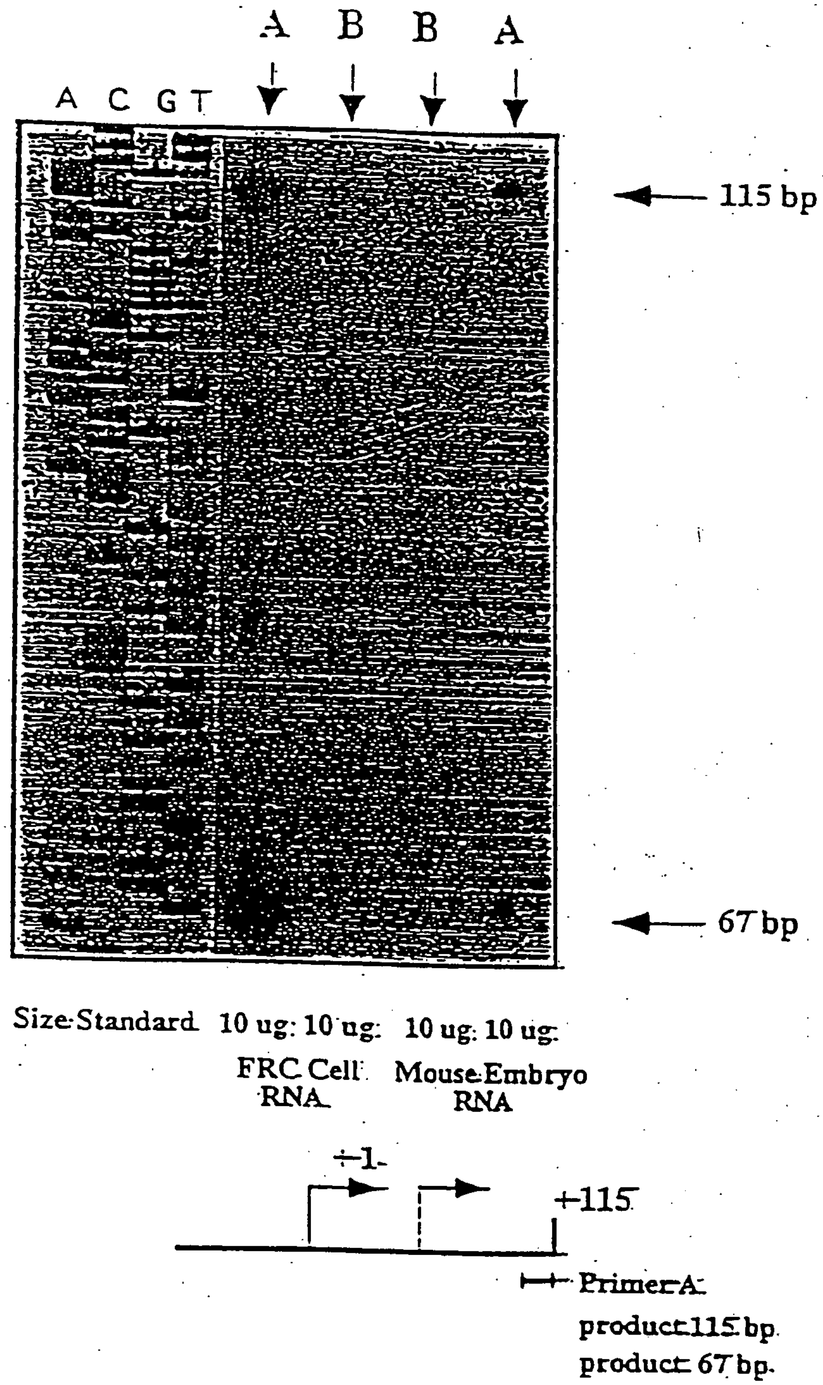

A B B A
A C G T
115 bp
67 bp
Size Standard 10 ug 10 ug 10 ug 10 ug
FRC Cell RNA
Mouse Embryo RNA
+1
+115
Primer A
product 115 bp
product 67 bp

FIGURE 2

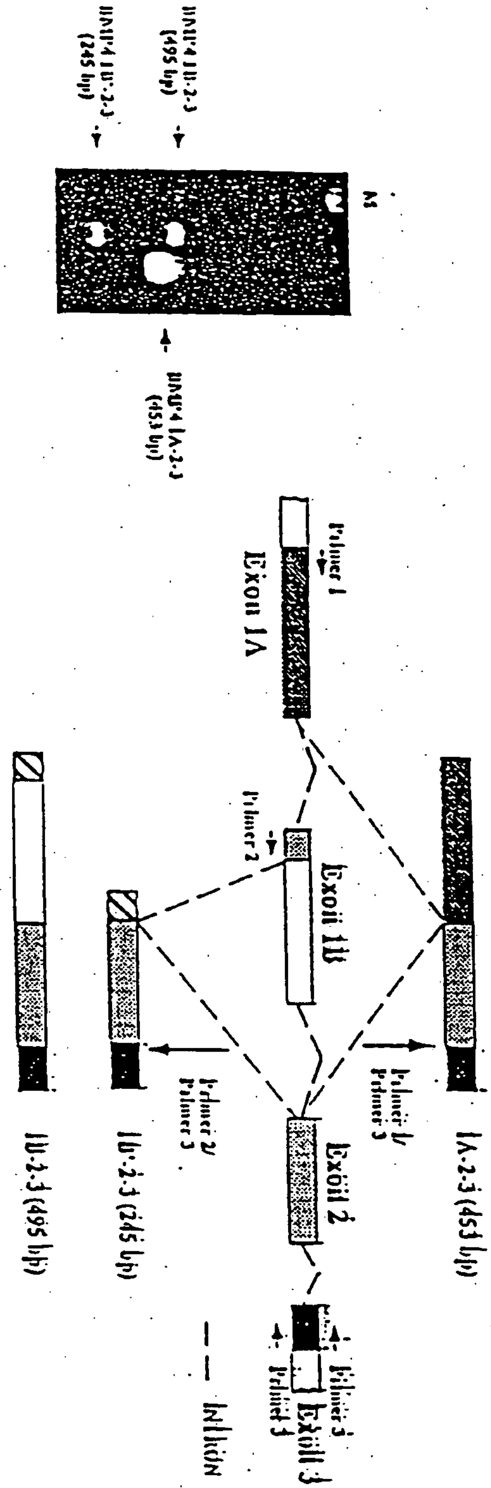
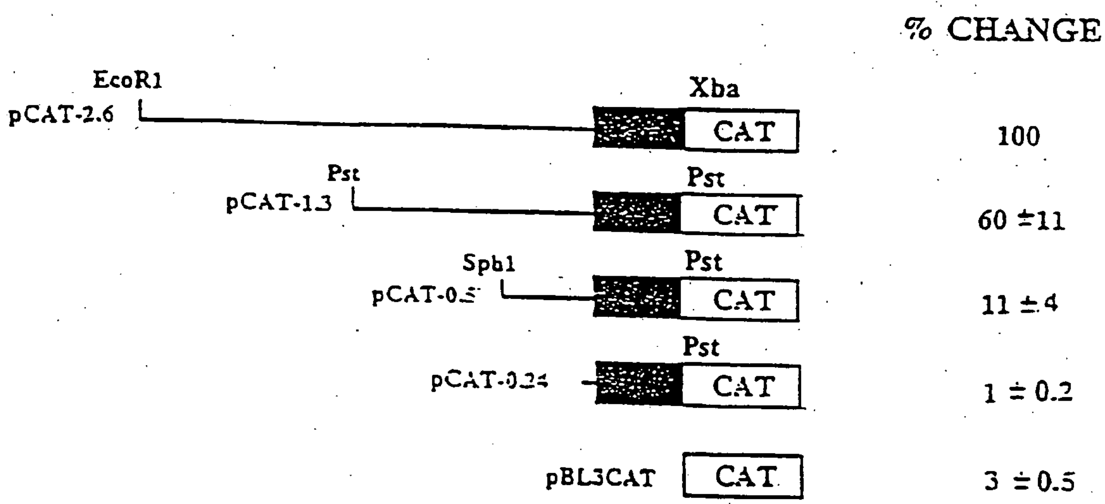

Exon 1A
Exon 1B
Exon 2
Exon 3
Intron
1A-2-3 (453 bp)
1B-2-3 (245 bp)
1B-2-3 (495 bp)

FIGURE 3B

A.


% CHANGE
EcoR1
Xba
pCAT-2.6
CAT
100
Pst
Pst
pCAT-1.3
CAT
60 ±11
Spb1
Pst
pCAT-0.5
CAT
11 ± 4
Pst
pCAT-0.24
CAT
1 ± 0.2
pBL3CAT
CAT
3 ± 0.5

B.

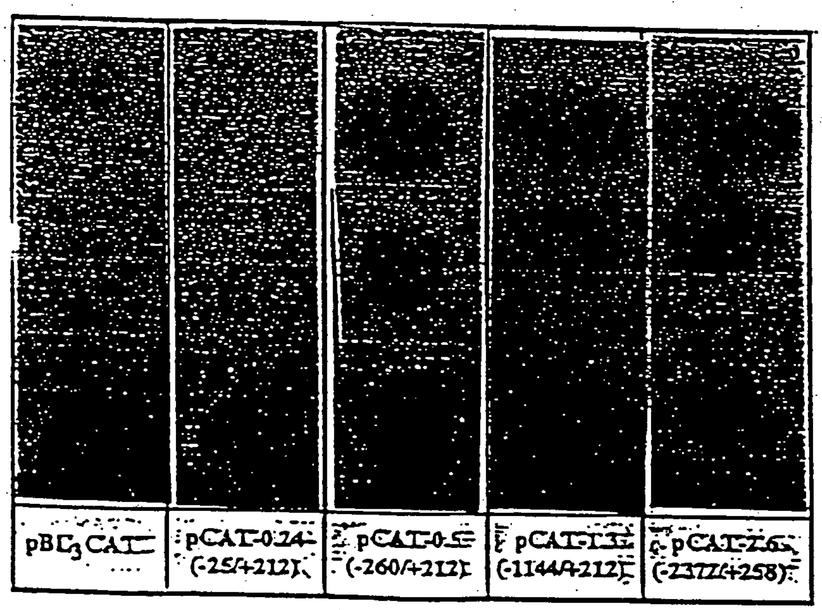

pBL3CAT
pCAT-0.24 (-25/+212)
pCAT-0.5 (-260/+212)
pCAT-1.3 (-1144/+212)
pCAT-2.6 (-2372/+258)

FIGURE 4

-2736

[illegible]

[illegible] -2647

[illegible] -2555

• -2484 Eco RV

[illegible] -2463

[illegible]

E-box 1 2

[illegible] -2375

3

[illegible] -2283

[illegible] -2191

[illegible] -2101

4

[illegible] -2011

Xba -1996 5 6

[illegible] -1921

E-box 2 7

[illegible] -1831

[illegible] -1739

E-box 3

[illegible] -1649

[illegible] -1559

Bsa B1 -1511

[illegible] -1469

9 E-box 4 CA Repeat

[illegible] -1377

E-box 5 9 10

[illegible] -1285

11 E-box 6 Bsa B1 -1232

[illegible] -1193

12 13

[illegible] -1101

E-box 7

[illegible] -1015

• BAM -1013

[illegible] -925

• BAM -876

[illegible] -837

[illegible] -747

[illegible] -659

[illegible] -573

[illegible] -481

[illegible] -397

E-box 8

[illegible] -307

[illegible] -247

[illegible] -130

[illegible] -40

P53 Zif268 -1 +1 E-box 9

[illegible] 47

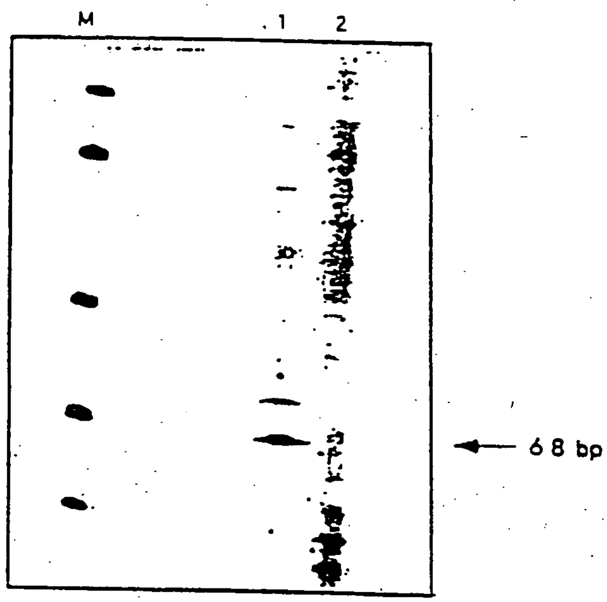
[illegible] 139

Candidate Homeo Box Binding Sites

- Homeobox 10 and 12 are identical at 8/8 sites, in an inverted orientation.
- Homeobox 3, 4, 5, 9 should bind MSX1 and/or MSX2 with relatively high affinity.

FIGURE 5

FIGURE 6A



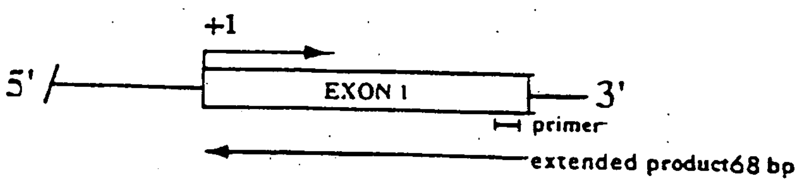


FIGURE 6B

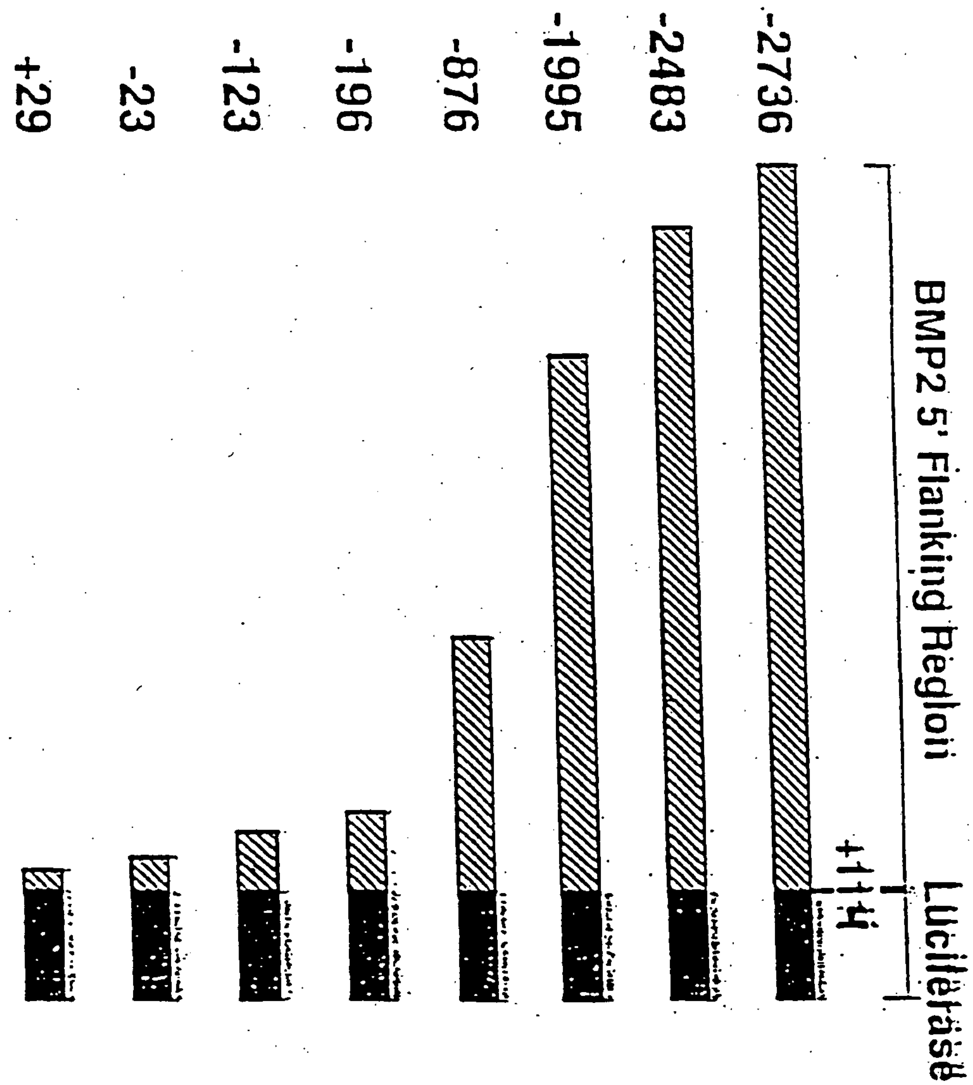

BMP2 5' Flanking Region
Luciferase
+114
-2736
-2483
-1995
-876
-196
-123
-23
+29

FIGURE 7

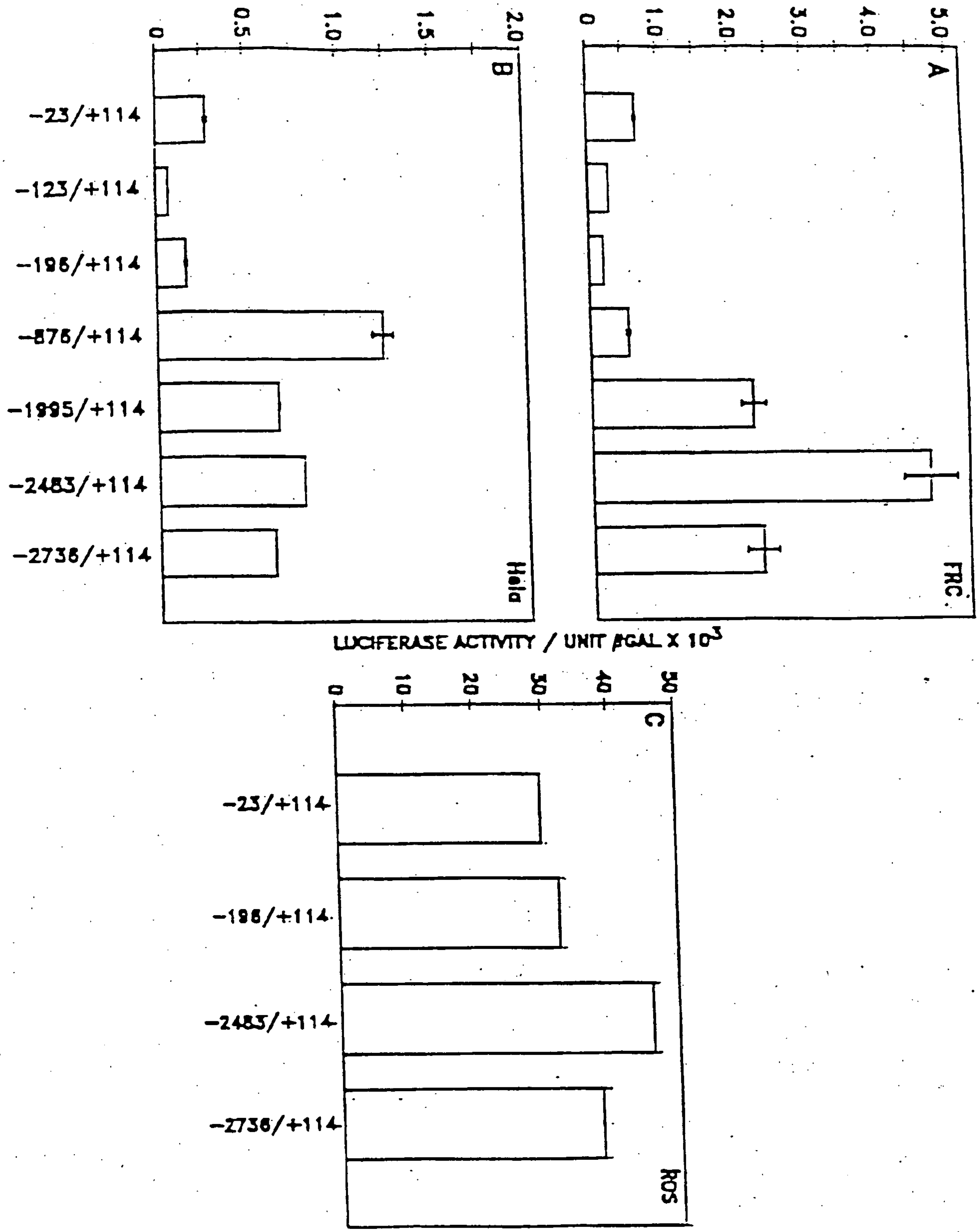

A
FRC
B
Hela
C
ROS
-23/+114
-123/+114
-198/+114
-878/+114
-1995/+114
-2483/+114
-2738/+114
LUCIFERASE ACTIVITY / UNIT βGAL X 10³

FIGURE 8

```
   1  GAATTCATTT AAGCTGGATT CACTTCTAGG TCCCATGCGT TTACACTCAT
  51  TTCCACCACA AGAGGGCAGC CATCTCTAAA AAAACAACAG TCGAGTGCTC
 101  TTCAGAGAAA TTGGGCCAAA CTTGAGGAAA GTTCCTGGGA AAGGCTTTTT
 151  AGCAGCACCT CTCTGGGCTA CAAAAAAGAA GCCAGCAGGC ACCACCAAGG
 201  TGGAGTAACT GTCCAGAGGC ATCCATTTTA CCTCAGAGAC TTGATTACTA
 251  AGGATATCCT AAACGGCCAA ACTCTCTCTT CTGGTGTTCC AGAGGCCCAA
 301  AGCTGCAAGG CATTGTTGAT GTCATCACCA AAGGTTTCAT TTTCATCTTT
 351  TCTTGGGGTT GGTCCAACAG CTGTCAGCTT TCTCTTCCTC ATTAAAGGCA
 401  ACTTTCTCAT TTAAATCTCA TATAGGTTCG GAGTTTCTTG CTTTGCTCCT
 451  TCCGCCTCCG CGATGACAGA AGCAATGGTT AACTTCTCAA TTAAACTTGA
 501  TAGGGAAGGA AATGGCTTCA GAGGCGATCA GCCCTTTTGA CTTACACACT
 551  TACACGTCTG AGTGGAGTGT TTTATTGCCG CCTTGTTTGG TGTCTCATGA
 601  TTCAGAGTGA CAACTTCTGC AACACGTTTT AAAAAGGAAT ACAGTAGCTG
 651  ATCGCAAATT GCTGGATCTA TCCCTTCCTC TCCTTTAATT TCCCTTGTAG
 701  ACAGCCTTCC TTCAAAAATA CCTTATTTGA CCTCTACAGC TCTAGAAACA
 751  GCCAGGGCCT AATTTCCCTC TGTGGGTTGC TAATCCGATT TAGGTGAACG
 801  AACCTAGAGT TATTTTAGCT AAAAGACTGA AAAGCTAGCA CACGTGGGTA
 851  AAAAAATCAT TAAAGCCCCT GCTTCTGGTC TTTCTCGGTC TTTGCTTTGC
 901  AAACTGGAAA GATCTGGTTC ACAACGTAAC GTTATCACTC TGGTCTTCTA
 951  CAGGAATGCT CAGCCCATAG TTTTGGGGGT CCTGTGGGTA GCCAGTGGTG
1001  GTACTATAAG GCTCCTGAAT GTAGGGAGAA ATGGAAAGAT TCAAAAAAGA
1051  ATCCTGGCTC AGCAGCTTGG GGACATTTCC AGCTGAGGAA GAAAACTGGC
1101  TTGGCCACAG CCAGAGCCTT CTGCTGGAGA CCCAGTGGAG AGAGAGGACC
1151  AGGCAGAAAA TTCAAAGGTC TCAAACCGGA ATTGTCTTGT TACCTGACTC
1201  TGGAGTAGGT GGGTGTGGAA GGGAAGATAA ATATCACAAG TATCGAAGTG
1251  ATCGCTTCTA TAAAGAGAAT TTCTATTAAC TCTCATTGTC CCTCACATGG
1301  ACACACACAC ACACACACAC ACACACACAC ACACATCACT AGAAGGGATG
1351  TCACTTTACA AGTGTGTATC TATGTTCAGA AACCTGTACC CGTATTTTTA
1401  TAATTTACAT AAATAAATAC ATATAAAATA TATGCATCTT TTTATTAGAT
1451  TCATTTATTT GAATATAAAT GTATGAATAT TTATAAAATG TAATAATGCA
1501  CTCAGATGTG TATCGGCTAT TTCTCGACAT TTTCTTCTCA CCATTCAAAA
1551  CAGAAGCGTT TGCTCACATT TTTGCCAAAA TGTCTAATAA CTTGTAAGTT
1601  CTGTTCTTCT TTTTAATGTG CTCTTACCTA AAAACTTCAA ACTCAAGTTG
1651  ATATTGGCCC AATGAGGGAA CTCAGAGGCC AGTGGACTCT GGATTTGCCC
1701  TAGTCTCCCG CAGCTGTGGG CGCGGATCCA GGTCCCGGGG GTCGGCTTCA
1751  CACTCATCCG GGACGCGACC CCTTAGCGGC CGCGCGCTCG CCCCGCCCCG
1801  CTCCACCGCG GCCCCGTACG CGCCGTCCAC ACCCCTGCGC GCCCGTCCCG
1851  CCCGCCCGGG GGATCCCGGC CGTGCTGCCT CCGAGGGGGA GGTGTTCGCC
1901  ACGGCCGGGA GGGAGCCGGC AGGCGGCGTC TCCTTTAAAA GCCGCGAGCG
1951  CGCGCCAGCG CGGCGTCGTC GCCGCCGGAG TCCTCGCCCT GCCGCGCAGA
2001  GCCCTGCTCG CACTGCGCCC GCCGCGTGCG CTTCCCACAG CCCGCCCGGG
2051  ATTGGCAGCC CCGGACGTAG CCTCCCCAGG CGACACCAGG CACCGGAGCC
2101  CCTCCCGGCG AAAGACGCGA GGGTCACCCG CGGCTTCGAG GGACTGGCAC
2151  GACACGGGTT GGAACTCCAG ACTGTGCGCG CCTGGCGCTG TGGCCTCGGC
2201  TGTCCGGGAG AAGCTAGAGT CGCGGACCGA CGCTAAGAAC CGGGAGTCCG
2251  GAGCACAGTC TTACCCTCAA TGCGGGGCCA CTCTGACCCA GGAGTGAGCG
2301  CCCAAGGCGA TCGGGCGGAA GAGTGAGTGG ACCCCAGGCT GCCACAAAAG
2351  ACACTTGGCC CGAGGGCTCG GAGCGCGAGG TCACCCGGTT TGGCAACCCG
2401  AGACGCGCGG CTGGACTGTC TCGAGAATGA GCCCCAGGAC GCCGGGGCGC
2451  CGCAGCCGTG CGGGCTCTGC TGGCGAGCGC TGATGGGGGT GCGCCAGAGT
2501  CAGGCTGAGG GAGTGCAGAG TGCGGCCCGC CCGCCACCCA AGATCTTCGC
2551  TGCGCCCTTG CCCGGACACG GCATCGCCCA CGATGGCTGC CCCGAGCCAT
2601  GGGTCGCGGC CCACGTAACG CAGAACGTCC GTCCTCCGCC CGGCGAGTCC
2651  CGGAGCCAGC CCCGCGCCCC GCCAGCGCTG GTCCCTGAGG CCGACGACAG
2701  CAGCAGCCTT GCCTCAGCCT TCCCTTCCGT CCCGGCCCCG CACTCCTCCC
2751  CCTGCTCGAG GCTGTGTGTC AGCACTTGGC TGGAGACTTC TTGAACTTGC
```

FIGURE 9A

```
2801  CGGGAGAGTG ACTTGGGCTC CCCACTTCGC GCCGGTGTCC TCGCCCGGCG
2851  GATCCAGTCT TGCCGCCTCC AGCCCGATCA CCTCTCTTCC TCAGCCCGCT
2901  GGCCCACCCC AAGACACAGT TCCCTACAGG GAGAACACCC GGAGAAGGAG
2951  GAGGAGGCGA AGAAAAGCAA CAGAAGCCCA GTTGCTGCTC CAGGTCCCTC
3001  GGACAGAGCT TTTTCCATGT GGAGACTCTC TCAATGGACG TGCCCCCTAG
3051  TGCTTCTTAG ACGGACTGCG GTCTCCTAAA GGTAGAGGAC ACGGGCCGGG
3101  GACCCGGGGT TGGCTGGCGG GTGACACCGC TTCCCGCCCA ACGCAGGGCG
3151  CCTGGGAGGA CTGGTGGAGT GGAGTGGACG TAAACATACC CTCACCCGGT
3201  GCACGTGCAG CGGATCCCTA GAGGGGTTAG GCATTCCAAA CCCCAGATCC
3251  CTCTGCCTTG CCCACTGGCC TCCTTCCTCC AGCCGGTTCC TCCTCCCCAA
3301  GTTTTCGATA CATTATAAGG GCTGTTTTGG GCTTTCAAAA AAAAAAATGC
3351  AGAAATCCAT TTAAGAGTAT GGCCAGTAGA TTTTACTAGT TCATTGCTGA
3401  CCAGTAAGTA CTCCAAGCCT TAGAGATCCT TGGCTATCCT TAAGAAGTAG
3451  GTCCATTTAG GAAGATACTA AAAGTTGGGG TTCTCCATGT GTGTTTACTG
3501  ACTATGCGAA TGTGTCATAG CTTACACGTG CATTCATAAA CACTATCTAT
3551  TTAGTTAATT GCAGGAAGGT GCATGGATTT CTTGACTGCA CAGGAGTCTT
3601  GGGGAAGGGG GAACAGGGTT GCCTGTGGGT CAACCTTAAA TAGTTAGGGC
3651  GAGGCCACAA CTTGCAAGTG GCGTCATTAG CAGTAATCTT GAGTTTAGCG
3701  CTTACTGAAT CTACAAGTTT GATATGCTCA ACTACCAGGA AATTGTATAC
3751  AGCGCCTCTA AGGAAGTCAC TTGTGCATTT GTGTCTGTTA ATATGCACAT
3801  GAGGCTGCAC TGTATAAGTT TGTCAGGGAT GCAGTGTCCG ACCAACCTAT
3851  GGCTTCCCAG CTTCCTGACA CCCGCATTCC CAGCTAGTGT CACAAGAAAA
3901  GGGTACAGAC GGTCAAGCTC TTTTTAATTG GGAGTTAAGA CCAAGCCCCA
3951  AGTAAGAAGT CCGGCTGGGA CTTGGGGGTC CTCCATCGGC CAGCGAGCTC
4001  TATGGGAGCC GAGGCGCGGG GGCGGCGGAG GACTGGGCGG GGAACGTGGG
4051  TGACTCACGT CGGCCCTGTC CGCAGGTCGA CCATGGTGGC CGGGACCCGC
4101  TGTCTTCTAG TGTTGCTGCT TCCCCAGGTC CTCCTGGGCG GCGCGGCCGG
4151  CCTCATTCCA GAGCTGGGCC GCAAGAAGTT CGCCGCGGCA TCCAGCCGAC
4201  CCTTGTCCCG GCCTTCGGAA GACGTCCTCA GCGAATTTGA GTTGAGGCTG
4251  CTCAGCATGT TTGGCCTGAA GCAGAGACCC ACCCCCAGCA AGGACGTCGT
4301  GGTGCCCCCC TATATGCTAG ATCTGTACCG CAGGCACTCA GGCCAGCCAG
4351  GAGCGCCCGC CCCAGACCAC CGGCTGGAGA GGGCAGCCAG CCGCGCCAAC
4401  ACCGTGCGCA CGTTCCATCA CGAAGGTGAG CGGGCGGCGG GTGGCGGGGC
4451  GGGGACGGCG GGCGGGCGGA GACTAGGCGG GCAGCCCGGG CCTCCACTAG
4501  CACAGTAGAA GGCCTTTCGG CTTCTGTACG GTCCCCTCTG TGGCCCCAGC
4551  CAGGGATTCC CCGCTTGTGA GTCCTCACCC TTTCCTGGCA AGTAGCCAAA
4601  AGACAGGCTC CTCCCCCTAG AACTGGAGGG AAATCGAGTG ATGGGGAAGA
4651  GGGTGAGAGA CTGACTAGCC CCTAGTCAGC ACAGCATGCG AGATTTCCAC
4701  AGAAGGTAGA GAGTTGGAGC TCCTTAAATC TGCTTGGAAG CTCAGATCTG
4751  TGACTTGTGT TCACGCTGTA GTTTTAAGCT AGGCAGAGCA AGGGCAGAAT
4801  GTTCGGAGAT AGTATTAGCA AATCAAATCC AGGGCCTCAA AGCATTCAAA
4851  TTTACTGTTC ATCTGGGCCT AGTTTGAAAG ATTTCTGAAT CCCTATCTAA
4901  TCCCCGTGGG AGATCAATTC CACAATTCGT CATATTGTTT CCACAATGAC
4951  CTTCGATTCT TTGCTTAAAT CTTAAATCTC CAAGTGGAGA CAGCGCAACG
5001  CTTCAGATAA AAGCCTTTCT CCCACTGCCT GCTACCTTCC TAGGCAAGGC
5051  AATGGGGTTT TTAAACAAAT ATATGAATAT GATTTCCCAA GATAGAATAA
5101  TGTTGTTTAT TTCAGCTGAA ATTTCCTGGA TTAGAAAGGC TGTAGAGGCC
5151  TATTGAAGTC TCTTGCACCG ATGTTCTGAA AGCAGTTAGT AAAAAATCAT
5201  GACCTAGCTC AATTCTGTGT GTGCCACTTT CAATGTGCTT TTGACTTAAT
5251  GTATTCTCCA TAGAACATCA GTTCCTTCAA GTTCTAGAAG AATTCAGATT
5301  TAAAGTTTTG CTTTGCCTTG CTGAGGGGAT AAATTTTAAG TAGAAATCTA
5351  GGCTCTGAAA TGATAGCCCA ACCCCATCTC CAGTAAGGGA TGACTGACTC
5401  AAACCTTGAG AAGTCTGGGT GATAATAGGA AAAGTCCACA AGCAGGTCAC
5451  AGAGCGCGAG ATGGATCTGT CTTGAGGCAG CCAATGGTTA TGAAGGGCAC
5501  TGGAAATCCA TCTCTTTCAA ACTGGTGTCT AGGGCTTTCT GGGAGCAAAG
5551  CTTAGACCAC ATTCTGCTCC TCAAGGTTTG CCTACTGAAA GCAGGGAGAT
```

FIGURE 9B

5601 TCTGGGTGTT CACCCCCATC CTTCACCCCC AGGTGATTCT GGGCTTAGCT
5651 AATCTCTCCT GGTTAATATT CATTGGAAAG TTTTTATAGA TCAAAACAAA
5701 CAAACCTACT ATCCAGCACA GGTGTTTTTC CCACTGCCTC TGGAGATATA
5751 GCAAGAAAAC CATATATTCA TGTATTTCCT TATTAGTCTT TTCTAACGTG
5801 AAAATTATTC CTGACCTATA AAAAATGAAG GAGGTATTTT ATCTTAACTA
5851 AGCTAAAAGA ATCGCTTAAG TCAATTGAAA CTCAAAAATC CAATTGAATG
5901 AAAGGTTCGT CAATAAAAAT CTACATTTTT CTTACTCTTC CTTTGGAAAT
5951 AGCTTGATAA AAACACAGAC AAAACAAAGT CTGTGTGCTT ATTTGAAAAC
6001 TTAGTGAGCT TCAGTTCATA AGCAAAAAAT GTAGTTTAAA AGTGATTTTT
6051 CTGTTGTAAA ACGTGATAGA AGTTATTGAC TTGTTTAAAA TAAACTTGCA
6101 CTAACTTTAT ACCTTGGTGC AATTAGATGT AATGTTTACT GTAAATTTCA
6151 GGAAAACCAT TTTTTTTTTT TGGTCATGAT CAGGTACACA TGGCATTTGG
6201 GAAGACTTTT CACATTGTTG AGTAACCTAG AGTTTGTTTG TTTGTTTGTT
6251 TGTTTTTAAG CATTCTTGTG CCACTAGAAA AACCTTAATA AGCCATGTGT
6301 TACTTGGTAG ACTTCTTCCT AAGTTCTAGA AAGTGGCTTA ATGCCACGAT
6351 GAGACAAAAC ATACCATAGT AGTCTTTCAA CCAGTGGCAG AGTCTTCCAG
6401 ACAAAATCTC CTGTTGAACA TTAAGACCAT GGATTTTTAT CCAGGAGAGC
6451 CCAGGCTTTG CTGAATCACC ACCCTCCAAC CCCACTCCAA GGTCACCGAA
6501 GGCCTCCCCA ACTGGCTGCC ATTGAGAAAC TGTTTGAAAT TGATTGACTC
6551 CATTGGCCCT ACAGAGACTT CTCCTTTAGT GGCAGATCAT ATACTGAAGG
6601 ATCCAAGCTT GCTCTTCTGA CTATGAAGAG CACAGTCTTT CTTTTTCTTT
6651 ATGGAATAAA CAAACTATGT GGCCCTGTGA CTAAAGTTTT CAAAGAGGGA
6701 GAGATCCTGT TAGCAGAAGT GCAACTGCCC AGAAACTAGC CACAGGCTAG
6751 GATATTCCAA AGTACAACTC TAAAGTATGG TCCATCCTAA ATTCTAGCAT
6801 GGGGTTGAAT ACCGGCATCC AGGAATACTT CTCTCTACCT CTGGCTATTG
6851 CAGTGAGATT ACGAAGACCC TGGGGGGAAA AACAGTTGCT TAGTTTACAG
6901 ATGTTCCTTG CCACAGATGT TCTCAGTATC TCTTGTTTGT CAGAGGATCC
6951 TTTCAATCCC TCTTGACATT TCCAATCTGC TTTTGTCCTC TCTACATGTG
7001 CCTTGTGGCA TTTCGCTTGG TCTTTAGAGA ATCCCTTTCT GGAGCTGCAG
7051 GTTCCCTTGT AGGATCTGTG TTCAGGAGAA CAGGGACCTT GGCAGGTTAG
7101 TGACAACTAC CAAACCCTGC TTTCCTTCCC TGCCACTTCC TTTGTTGCCT
7151 TAAAAATTAA ACCTTAACTC TCTGTGTCTA AACCTTTTCT TCTTCCTCTT
7201 TGTCATTTAC TTTATTTATT TGTCATGTAC TTTATCCTGT AGAAAATCAC
7251 AGTGTGGCCC AAAGCCCCTT GAATCTTGTT GCAGCGGTGA GATGCAGCTG
7301 CTGATCTGGA ATAGCCTTAG GCTGTGTGTT TGATCACAAT GCTTTCTGTC
7351 CAAAAGTGTG CAAATCCTCC AAGCTTAATG ATAACTTTTG AAATGAAACT
7401 CACCCTACTT TAGGGCAAAC AAGTAGCCAC AGAGAGCAGG ATCTAAACAA
7451 GGTCTGGTGT CCCATTTGGC TGTGTCCCTT CAATTTTCTG TTCATTTAGC
7501 TCTGTCTGCA TCTAAAGGGT GCTGGGCAAT AAGTTTTGAT CTTCAGGGCA
7551 AAACTCAATC TTCAGTTACC ATGGTATCAG GTACCAATTC CTAGTGATTT
7601 GTGCTATGGC TTAGGATTTG ATTTCTCTCC TACATTAGGT AATATCTTTC
7651 AATGGCTAGA ACTTGGGCAT TGCAGTACAC TCAAGTTAAC AGTTCTGTGA
7701 CCTAAGGAAG TCACATAACC TCTCTGAATT CTCTACTGTT TCATTCACAA
7751 AATGGAGAAA ATCATGGCTC TTTCTTAATG TGCGAATTCA TAGAAAGGTG
7801 ATGACACCAG ATTTGGCAGA AGGAAGGAAA GGAAGGAAGG AAGAAAGAAA
7851 GAAAGAAAGA AAGAAAGAAA GAAAGAAAGA AAGAAAGAAA GGAAGGAAGG
7901 GAGAGAGAGA GAAGGGAAGG GAAAGGGAAA GGGAAAGGAA AGAAAAGAAA
7951 GGAAGGAAGA AAAGGAAGGA AGGAAGGAAA GAAGGAAGGA AGGAAAAGAA
8001 AGAGAAGAAA GCATTCAGCA TATGAACTAA TGTTTCCTGG TGACTTTTTA
8051 TATCATATCC TTGTTCTAGG AAGTGGCCCT AGCCATATCT TTTGGGTTAT
8101 TTTGAGGTAG AGGATAATCA ACATAGTGTA GAACATTAAA TCTGGGTTTT
8151 GTTTCTAGAA GAGGCTAGAA TGGCATGGCT GTCCCACTTG CTCCTCTTTC
8201 AGGCAGTATG GCAGCCACCA TTCTCTCTGT AAGATCTAGG AGGCTGACAC
8251 TCAGGTTGGA GACAGGTCAG AATCCTGAAA TCACTTAGCA AGTTCAGCTG
8301 ATTCAACAAG GGATATTTAC AGAGAATTAA CAGCTATTCC AGCTTCCAAA
8351 AAGTGTACAT TACCTACTCT GTATTTTCAG AACCCCAGGT TTGCTGTGAT

FIGURE 9C

8401 AATTTGGTAG AAGCCTTTTC CTGTAATTTT CTTTATTTAA AAGATATTTT
8451 CATTTTCCAC CCTCAAGAAG AGGTTGAAAC TTGTCCCTTG AAGTAGAAGA
8501 GGTGTTGTGT GTCCTGACCC TGAGGAAGTT GGCCTTGTTG AGGTCTTCTG
8551 TAAATTCTTG AATTCTCTGT ATAATTTCAA TGAATAGTCA TGTTTGATAC
8601 CTTGGTATAA AGGATGGGAT AAGATCTTTC AAGGCTTAGG CTGATGGAAA
8651 CGCTGCTGAA AGACTAGAGA TTGCTCTTTC CTTTGGCATC TGTCTTGGGT
8701 AGTAATATTG TTCTCTGTGA AGGCCCACTT ATTCTGTCTT GAAAATTCTT
8751 CTTACCTCCA GAGTGATAGG CCACAGGGAG TACTGTTTCT ATGTTTGCAG
8801 TTGAAAGATG ACAATTTCAT ATGGTCCAAA CTTGGCTTTA TTTCTTGGTG
8851 AGATATTATT CTGTTACTTC AATGACCTGT CTCCATTATT TATCTTGAGG
8901 CTCACCTCTT CCCTTTTGTT GACTGTTGTG CAATTTGTGG AAGGCCCTGG
8951 GTAGTCAGCC TTTATACTCT GTCTGTACAG GAAATAAAGT GCATGTCACC
9001 ATGCCAAAGT CAGGAGATGC CGGTGTGATT AGGGTCCACG GGATTTTGCT
9051 ACTGTTTTTA TTTCTATCGA TGAATTGCCT TAGGCAGAAA CATTAAGGGA
9101 CACCAGAATG GTGATGAAAG GCTTTTTATA ACAGAAGCTA AATGCAGTCC
9151 TTCATACTTC ATGGAATGCC CCTGTCCTAA AGTACCATTA ACCGATAGTG
9201 GAGTCAGAAC ATAAATGGCT CCCCAAAGGT ATCACCAAGA ACTTTTGGCA
9251 AACAGATGCA AGAGGATTAT GAAGAATCGC AGCTTGGTCT GGTAATCTTC
9301 CTGTTGCAAA GAGAAGAGCT TTAGAAGACC CCCCTTGAGT CCCTGGCTGG
9351 CTTAACATAG CATGAACCCT CATGTGTTGG CCAACATTAA GGCTTTTTCT
9401 ATAAAAGTCT CCTCCTTCAT CAGTATACGC TCGAGTATGA AAAGCATCCT
9451 TTTAAACCTT GACTCTGTGT GGTCCAGAAA CAGCAGCATC CCTTGCTTAA
9501 GAGCTTAATG GAGATGCAGG AGTGCAGGCC TCTTCCCAGA CCGGCTGATG
9551 TGCAGGTCAA AGTCTAAGCA CTGCTGGATC AACACAGAAG TTATTCCGAA
9601 TGAGGATGAG ATGGATACGA GAGAACAGGA AGTAGGAAGG GATTTCTTTA
9651 TCGTGAATTG CTACAGCAGC CTAATGTCAC CCCATACCCT TCTGAAGAAC
9701 TATGTCCCTG TGGATGCCTT TGTCTCTAGA GTTCTGAGCA AAATGGTAGG
9751 GTGTGCTTTG CAAAATGTCA TCATTGATGT TGAATTTCAA AGTCTTTAAT
9801 TAAGGGGCTG AAATCTGTAT ATTGAGATTT GTAAATCATC TAAATTGTAG
9851 AGTAATGTTT GCACAGGCTG CTTAAGGGAT TGACATTAAA GCTCGTTTTC
9901 TTAGTTAAGA AATACAGTCA TTTCCTCAAC TCCTCAGTCA TTAGCTCTCT
9951 ACTAAGTACA GTGCTGACTT TTTTAAAATT AAAGTCTGTG AATTCCAAAG
10001 AAGTGTTTCA CTATTTCCTC CATTATTATA GCTACCTAGA AGCTATGTTC
10051 ATATATTGGA TTAAAAACGT AGCAATTACA AAGTTAATGT GGCCATATAG
10101 AAAAGGGAAA AGAAACTCCG CTTTCACTTT AATATATATA TGTGTGTGTG
10151 TATATCATAT ATATACATGT TGTGTGTGTA TATATATATA TATATATATA
10201 TATATATATA TATATATATA TATATATATA TGTTGTGTTA AGCAGTAAAC
10251 TCAGGCCATG GACAGAGGGG CAGACATTGT ATCTCTAGGC CTGACATTTT
10301 TAATTTCTGG TTGCAGGTTT TTATGTAGTT TAACTTAAAC CATGCACTGA
10351 AGTTTTAAAT GCTCGTAAGG AATTAAGTTA CCATTGGCTC TCTTACCAAA
10401 TGCGTTTCTT TTTTCTCTCC ACCCTGATCA AACTAGAAGC CGTGGAGGAA
10451 CTTCCAGAGA TGAGTGGGAA AACGGCCCGG CGCTTCTTCT TCAATTTAAG
10501 TTCTGTCCCC AGTGACGAGT TTCTCACATC TGCAGAACTC CAGATCTTCC
10551 GGGAACAGAT ACAGGAAGCT TTGGGAAACA GTAGTTTCCA GCACCGAATT
10601 AATATTTATG AAATTATAAA GCCTGCAGCA GCCAACTTGA AATTTCCTGT
10651 GACCAGACTA TTGGACACCA GGTTAGTGAA TCAGAACACA AGTCAGTGGG
10701 AGAGCTTCGA CGTCACCCCA GCTGTGATGC GGTGGACCAC ACAGGGACAC
10751 ACCAACCATG GGTTTGTGGT GGAAGTGGCC CATTTAGAGG AGAACCCAGG
10801 TGTCTCCAAG AGACATGTGA GGATTAGCAG GTCTTTGCAC CAAGATGAAC
10851 ACAGCTGGTC ACAGATAAGG CCATTGCTAG TGACTTTTGG ACATGATGGA
10901 AAAGGACATC CGCTCCACAA ACGAGAAAAG CGTCAAGCCA AACACAAACA
10951 GCGGAAGCGC CTCAAGTCCA GCTGCAAGAG ACACCCTTTG TATGTGGACT
11001 TCAGTGATGT GGGGTGGAAT GACTGGATCG TGGCACCTCC GGGCTATCAT
11051 GCCTTTTACT GCCATGGGGA GTGTCCTTTT CCCCTTGCTG ACCACCTGAA
11101 CTCCACTAAC CATGCCATAG TGCAGACTCT GGTGAACTCT GTGAATTCCA
11151 AAATCCCTAA GGCATGCTGT GTCCCCACAG AGCTCAGCGC AATCTCCATG

FIGURE 9D

11201 TTGTACCTAG ATGAAAATGA AAAGGTTGTG CTAAAAAATT ATCAGGACAT
11251 GGTTGTGGAG GGCTGCGGGT GTCGTTAGCA CAGCAAGAAT AAATAAATAA
11301 ATATATATAT TTTAGAAACA GAAAAAACCC TACTCCCCCT GCCTCCCCCC
11351 CAAAAAAACC AGCTGACACT TTAATATTTC CAATGAAGAC TTTATTTATG
11401 GAATGGAATG AAAAAAACAC AGCTATTTTG AAAATATATT TATATCGTAC
11451 GAAAAGAAGT TGGGAAAACA AATATTTTAA TCAGAGAATT ATTCCTTAAA
11501 GATTTAAAAT GTATTTAGTT GTACATTTTA TATGGGTTCA ACTCCAGCAC
11551 ATGAAGTATA AGGTCAGAGT TATTTTGTAT TTATTTACTA TAATAACCAC
11601 TTTTTAGGGA AAAAAGATAG TTAATTGTAT TTATATGTAA TCAGAAGAAA
11651 TATCGGGTTT GTATATAAAT TTTCCAAAAA AGGAAATTTG TAGTTTGTTT
11701 TTCAGTTGTG TGTATTTAAG ATGCAAAGTC TACATGGAAG GTGCTGAGCA
11751 AAGTGCTTGC ACCACTTGCT GTCTGTTTCT TGCAGCACTA CTGTTAAAGT
11801 TCACAAGTTC AAGTCCAAAA AAAAAAAAAA AGGATAATCT ACTTTGCTGA
11851 CTTTCAAGAT TATATTCTTC AATTCTCAGG AATGTTGCAG AGTGGTTGTC
11901 CAATCCGTGA GAACTTTCAT TCTTATTAGG GGGATATTTG GATAAGAACC
11951 AGACATTACT GATCTGATAG AAAACGTCTC GCCACCCTCC CTGCAGCAAG
12001 AACAAAGCAG GACCAGTGGG AATAATTACC AAAACTGTGA CTATGTCAGG
12051 AAAGTGAGTG AATGGCTCTT GTTCTTTCTT AAGCCTATAA TCCTTCCAGG
12101 GGGCTGATCT GGCCAAAGTA CTAAATAAAA TATAATATTT CTTCTTTATT
12151 AACATTGTAG TCATATATGT GTACAATTGA TTATCTTGTG GGCCCTCATA
12201 AAGAAGCAGA AATTGGCTTG TATTTTGTGT TTACCCTATC AGCAATCTCT
12251 CTATTCTCCA AAGCACCCAA TTTTCTACAT TTGCCTGACA CGCAGCAAAA
12301 TTGAGCATAT GTTTCCTGCC TGCACCCTGT CTCTGACCTG TCAGCTTGCT
12351 TTTCTTTCCA GGATATGTGT TTGAACATAT TTCTCCAAAT GTTAAACCCA
12401 TTTCAGATAA TAAATATCAA AATTCTGGCA TTTTCATCCC TATAAAAACC
12451 CTAAACCCCG TGAGAGCAAA TGGTTTGTTT GTGTTTGCAG TGTCTACCTG
12501 TGTTTGCATT TTCATTTCTT GGGTGAATGA TGACAAGGTT GGGGTGGGGA
12551 CATGACTTAA ATGGTTGGAG AATTCTAAGC AAACCCCAGT TGGACCAAAG
12601 GACTTACCAA TGAGTTAGTA GTTTTCATAA GGGGGCGGGG GGAGTGAGAG
12651 AAAGCCAATG CCTAAATCAA AGCAAAGTTT GCAGAACCCA AGGTAAAGTT
12701 CCAGAGATGA TATATCATAC AACAGAGGCC ATAGTGTAAA AAAATTAAAG
12751 AATGTCTGAT CAGCGTCTCA GCACATCTAC CAATTGGCCA GATGCTCAAA
12801 CAGAGTGAAG TCAGATGAGG TTCTGGAAAG TGAGTCCTCT ATGATGGCAG
12851 AGCTTTGGTG CTCAGGTTGG AAGCAAAACC TAGGGAGGGA GGGCTTTGTG
12901 GCTGTTTGCA GATTGGGGAA TCCAGTGCTA GTTCCTGGCA GGGTTTCAGG
12951 TCAGTTTCCG GAGTGTGTGT CCTGTAGCCC TCCGTCATGG TTGAAGCCCA
13001 GGTCTCACCT CCTCTCCTGA CCCGTGCCTT AGAACTGACT TGGAAAGCGG
13051 TGTGCTTACA GCAAGACAGA CTGTTATAAT TAAATTCTTC CCAAGGACCT
13101 CCGTGCAATG ACCCCAAGCA CACTTACCTT CGGAAACCTT AAGGTTCTGA
13151 AGATCTTGTT TTAAATGACT ACCCTGGTTA GCTTTTGATG TGTTCCTTAT
13201 CCCTTTAGTT GTTGCACAGG TAGAAACGAT TAGACCCAAC TATGGGTAGC
13251 CTTGTCCTCC TGGTCCTTCA GTCATTCTCT AATGTCTCTT GCTTGCCATG
13301 GGCACTGTAA CAAACTGCAA TCTTAACATC TTATAAAATG AATGAACCAC
13351 ATATTTACAT CTCCAAGTCC TCCAGATGGG AGTGCGATCA TTCCATAAGG
13401 ATCCCACCTT CTGGCAGGTC TATCCAGTAC ATATTTTATG CTTCATTGGT
13451 CTTGATTTTC TTGGCTAAAA TTACTTGTAG CACAGCAGGC CCCATGTGAC
13501 ATATAGGTAT ATACATACAT GTATGTGCAT ATAGTGTGTA CATGTTCTAA
13551 TTTATACATA GCTATGTGAA GATTATGTTA CATATGTAGA TGGTCGCACT
13601 TCTGATTTCC ATTTAGGTTC AGAGAGAGAC GTCACAGTAA ATGGAGCTAT
13651 GTCATTGGTA TATCCCCGAG TGGTTCAGGT GTTCTCTCTA TTTTTTTAAG
13701 ATGGAGAACA CTCATCTGTA CTATCGAAAA CTGAGCCAAA TCACTTAGCA
13751 AATTTCTAGT CACTGCCTTG CTGTTAAGAT ACTGATTCAC TGGGTGCTGA
13801 CATGCTGAGC CCTGCCTACT TTTGCATGAA GGACAAGGAA GAGAGCTTGC
13851 AGTTAAGAAT GGTATATGTG GGGCTAGGGG GCGGCGTATA GACTGGCATA
13901 TATGTGAAGG AAGGTCACAA ACAGCCTGCA CTAATTTCCC TTTTCTGGTT
13951 TTATGTCTTG GCAGGGGAAA GGACAGGTAG GGTGGGGTTG AGGGGGAGGG

FIGURE 9E

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14001 CACACACATC TACTTGGATA AATTGCATCT CCTCTTTCCT TCACCCCGCC
14051 ACCATATCTT AAAGCCTTAT GACATCCTCT AGGGCAGAAT TTTCTCACCA
14101 GCTCCCCGCC CTACCAACTT CAAAGTGAAC TTCTAACTAA CTTGAGGGGC
14151 CAAAGTTCTA AATAAAACTT GTTAGAGTTT AGCGGGCACC TCAGTCATCA
14201 GGAATGCCTC CAGGAAAGCA AAAAGCTTGA TGTGTGTACA GCCACGTGGT
14251 GGAGTCCTGC CACCCTATGA TTCCTGTCCC AGTGGTCGTG TGGGGCCTGA
14301 GATCCTGAAT TTCTAATGAG CTCCCAGTAC GCCCTGACTC ACTGTGCCAG
14351 AGGACTGCAG TTTGAGTAGC AAGGTTGTGT GACTGTCTTC GATCATGGCT
14401 ACAGAAGCTG GCTCAAGTAC AGCCCTTCGT GTGTAAAAGC CATGTGTAAA
14451 TGAGAAGAAA CAGAAGGCAA AGCTGCGTTG CATGGCATCT GAATCAGTGC
14501 CCTGCAGTTT TGTTTTTTGT TTTTTTTTTT TCAAAGACAT TCTTTTTCCC
14551 AACAAGATGA GTGGCAATCT TATGTTCTAG CCACTCTTAG ACATGAAAAC
14601 ACTGGGTTGC TTATCTTGTA AAATCTGCTC TGCTTGCTTG CTTGGGCACG
14651 CTGCAGTCAG TTTAGTCAAA TGCGTGTCAG TACATCTATA TGTATGAGGG
14701 AGCAGGTGCA AGTCCTTAGA AATGTACTTT AAAAAACTTG AACACTTAAG
14751 TCAGTGTGCT GAGCTGCTCC TGTGTGATGT TAGGCCAAGC ACCTGAGTTA
14801 AAGGGATCTC TTTGAAGGCA GAGGGTAGAT GTCGTATGGT TGAAGCATTT
14851 GTTTATACTA AAATGATGCT TGACTTTTTT TCTAAGTTAT AAGACAGTAC
14901 ACTGTATAAG TTCATTGAAC CTAGAGGGTG GCATAGGACT CCAAATCTGG
14951 TATGGGAGGT TTGTTCTAAT GGAAGTTCGA ATCTTTTTTG CAGTTGGCTT
15001 GGAATAAAGT GCTTATGTGA ATGGGCTTAA GCTAGGGAAA AAAATGGGTT
15051 TCCCTCTGCA AAGAGGGTCA GCACAGAAAT AACTTCCTGG CTTTGCTTGC
15101 ATGAATGCCA CTTGTTAGCA GATGCCCTGT GGGGATCCGA ATTC
```

FIGURE 9F

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1    GAATTCGCTA GGTAGACCAG GCTGGCCCAG AACACCTAGA GATCATCTGG
51   CTGCCTCTGT CTCTTGAGTT CTGGGGCTAA AGCATGCACC ACTCTACCTG
101  GCTAGTTTGT ATCCATCTAA ATTGGGGAAG AAAGAAGTAC AGCTGTCCCC
151  AGAGATAACA GCTGGGTTTT CCCATCAAAC ACCTAGAAAT CCATTTTAGA
201  TTCTAAATAG GGTTTGTCAG GTAGCTTAAT TAGAACTTTC AGACTGGGTT
251  TCACAGACTG GTTGGGCCAA AGGTCACTTT ATTGTCTGGG TTTCAGCAAA
301  ATGAGACAAT AGCTGTTATT CAAACAACAT TTGGGTAAGG AAGAAAAATG
351  AACAAACACC ACTCTCCCTC CCCCCGCTCC GTGCCTCCAA ATCCATTAAA
401  GGCAAAGCTG CACCCCTAAG GACAACGAAT CGCTGCTGTT TGTGAGTTTA
451  AATATTAAGG AACACATTGT GTTAATGATT GGAGCAGCAG TGATTGATGT
501  AGTGGCATTG GTGAGCACTG AATCCGTCCT TCAACCTGCT ATGGGAGCAC
551  AGAGCCTGAT GCCCCAGGAG TAATGTAATA GAGTAATGTA ATGTAATGGA
601  GTTTTAATTT TGTGTTGTTG TTTTAAATAA TTAATTGTAA TTTTGGCTGT
651  GTTAGAAGCT GTGGGTACGT TTCTCAGTCA TCTTTTCGGT CTGGTGTTAT
701  TGCCATACCT TGATTAATCG GAGATTAAAA GAGAAGGTGT ACTTAGAAAC
751  GATTTCAAAT GAAAGAAGGT ATGTTTCCAA TGTGACTTCA CTAAAGTGAC
801  AGTGACGCAG GGAATCAATC GTCTTCTAAT AGAAAGGGCT CATGGAGACC
851  TGAGCTGAAT CTTTCTGTTC TGGATGAGAG AGGTGGTACC CATTGGAATG
901  AAAGGACTTA GTCAGGGGCA ATACAGTGTG CTCCAAGGCT GGGGATGGTC
951  AGGATGTTGT GCTCAGCCTC TAACACTCCT TCCAACCTGA CATTCCTTCT
1001 CACCCTTTGT CTCTGGCCAG TAGAATACAG GAACTCGTTC CTGTTTTTTT
1051 TTTTTTAAAT TCTGAAGGTG TGTAAGTACA AAGGTCAGAT GAGCGGCCCT
1101 AGGTCAAGAC TGCTTTGTGG TGACAAGGGA GTATAACACC CACCCCAGAA
1151 ACCAAGAACC GGAAATTGCT ATCTTCCAGC CCTTTGAGAG CTACCTGAAG
1201 CTCTGGGCTG CTGGCCTCAC CCCTTCCCTG CAGCTTTCCC TTTAGCAGAG
1251 GCTGTGATTT CCTTCAGCGC TTGGGCAAAT ACTCTTAGCC TGGCTCACCT
1301 TCCCCATCCT CGTTTGTAAA AACAAAGATG AAGCTGATAG TTCCTTCCCA
1351 GCTCCATCAG AGGCAGGGTG TGAAATTAGC TCCTGTTTGG GAAGGTTTAA
1401 AAGCCGGCCA CATTCCACCT CCCAGCTAGC ATGATTACCA ACTCTTGTTT
1451 CTTACTGTTG TTATGAAAGA CTCAATTCCT CATCTCCCTT TCCCTTCTTT
1501 TAAAAAGGGG CCAAAGGGCA CTTTGTTTTT TTCTCTACAT GGCCTAAAAG
1551 GCACTGTGTT ACCTTCCTGG AAGGTCCCAA ACAAACAAAC AAACAAACAA
1601 AATAACCATC TGGCAGTTAA GAAGGCTTCA GAGATATAAA TAGGATTTTC
1651 TAATTGTCTT ACAAGGCCTA GGCTGTTTGC CTGCCAAGTG CCTGCAAACT
1701 ACCTCTGTGC ACTTGAAATG TTAGACCTGG GGATCGATG GAGGGCACCC
1751 AGTTTAAGGG GGGTTGGTGC AATTCTCAAA TGTCCACAAG AAACATCTCA
1801 CAAAAACTTT TTTGGGGGGA AAGTCACCTC CTAATAGTTG AAGAGGTATC
1851 TCCTTCGGGC ACACAGCCCT GCTCACAGCC TGTTTCAACG TTTGGGAATC
1901 CTTTAACAGT TTACGGAAGG CCACCCTTTA AACCAATCCA ACAGCTCCCT
1951 TCTCCATAAC CTGATTTTAG AGGTGTTTCA TTATCTCTAA TTACTCGGGG
2001 TAAATGGTGA TTACTCAGTG TTTTAATCAT CAGTTTGGGC AGCAGTTATT
2051 CTAAACTCAG GGAAGCCCAG ACTCCCATGG GTATTTTTGG AAGGTACAGA
2101 GACTAGTTGG TGCATGCTTT CTAGTACCTC TTGCATGTGG TCCCCAGGTG
2151 AGCCCCGGCT GCTTCCCGAG CTGGAGGCAT CGGTCCCAGC CAAGGTGGCA
2201 ACTGAGGGCT GGGGAGCTGT GCAATCTTCC GGACCCGGCC TTGCCAGGCG
2251 AGGCGAGGCC CCGTGGCTGG ATGGGAGGAT GTGGGCGGGG CTCCCCATCC
2301 CAGAAGGGGA GGCGATTAAG GGAGGAGGGA AGAAGGGAGG GGCCGCTGGG
2351 GGGAAAGACT GGGGAGGAAG GGAAGAAAGA GAGGGAGGGA AAAGAGAAGG
2401 AAGGAGTAGA TGTGAGAGGG TGGTGCTGAG GGTGGGAAGG CAAGAGCGCG
2451 AGGCCTGGCC CGGAAGCTAG GTGAGTTCGG CATCCGAGCT GAGAGACCCC
2501 AGCCTAAGAC GCCTGCGCTG CAACCCAGCC TGAGTATCTG GTCTCCGTCC
2551 CTGATGGGAT TCTCGTCTAA ACCGTCTTGG AGCCTGCAGC GATCCAGTCT
2601 CTGGCCCTCG ACCAGGTTCA TTGCAGCTTT CTAGAGGTCC CCAGAAGCAG
2651 CTGCTGGCGA GCCCGCTTCT GCAGGAACCA ATGGTGAGCA GGGCAACCTG
2701 GAGAGGGGCG CTATTCTGAG GATTCGAGGT GCACCCGTAG TAGAAGCTGG
2751 GGATGGGGCT CAGGCTGTAA CCGAGGCAAA AGTTGGCCTA TTCCTCCTTC
```

FIGURE 10A

2801	CTTCTCCAAC	AGTGTTGGAG	GTGGGATGAT	GGAGGCTAAA	AGGCACCTCC
2851	ATATATGTTA	CTGCGTCTAT	CAACCTACTT	TAGGGAGGTG	CGGGCCAGGA
2901	GAGGCGGGAA	GGAGAGAAGG	CCTTGGAAGA	GAGGTCATTG	GGAAGAACTG
2951	TGGGGTTTGG	TGGGTTTGCT	TCCACTTAGA	CTATAAGAGT	GGGAGAGGAG
3001	GGAGTCAACT	CTAAGTTTCA	ACACCAGTGG	GGGACTGAGG	ACTGCTTCAT
3051	TAGGAGAGAG	AACCTAGCCA	GAGCTAGCTT	TGCAAAAGAG	GCTGTAGTCC
3101	TGCTTTGCTC	TAAAGCGCGA	CCCGGGATAG	AGAGGCTTCC	TTGAGCGGGG
3151	TGTCACCTAA	TCTTGTCCCC	AACGCACCCC	CTCCCAGCCC	CTGAGAGCTA
3201	GCGAACTGTA	GGTACACAAC	TCGCTCCCAT	CTCCAGGAGC	TATTTTCTTA
3251	GACATGGGCA	CCCATGATTC	TGCCTTCTGG	TACTCTCCCC	TCCCTGGGAA
3301	AGGGGTGTAA	GGTTCCGACG	GAACCGTGGC	CAGGATGCCG	AAAGGCTACC
3351	TGTGCGGGTC	TTCTGCCATG	CTGTGTCTGT	GCGGACATGC	CAGCAGGGCT
3401	AATGAGGAGC	TTGCGATACT	CCAAAGGGTT	CGGGAATTGC	GGGGTCCTTA
3451	CACGCAGTGG	AGTTGGGCCC	CTTTTACTCA	GAAGGTTTCC	GCCACGGCTT
3501	TGGTTGATAG	TTTTTTTAGT	ATCCTGGTTT	ATGAACTGAA	GGTTTTGTGA
3551	GATGTTGAAT	CACTAGCAGG	GTCATATTTG	GCAAACCGAG	GCTACTATTA
3601	AATTTTGGTT	TTAGAAGAAG	ATTCTGGGGA	GAAAGTGAAG	GGTAACTGCC
3651	TCCAGGAGCT	GTATCAACCC	CATTAAGAAA	AAAAAAAATA	CCAGGAGATG
3701	AAAATTTACT	TTGATCTGTA	TTTTTTAATT	AAAAAAAATC	AGGGAAGAAA
3751	GGAGTGATTA	GAAAGGGATC	CTGAGCGTCG	GCGGTTCCAC	GGTGCCCTCG
3801	CTCCGCGTGC	GCCAGTCGCT	AGCATATCGC	CATCTCTTTC	CCCCTTAAAA
3851	GCAAATAAAC	AAATCAACAA	TAAGCCCTTT	GCCCTTTCCA	GCGCTTTCCC
3901	AGTTATTCCC	AGCGGCGACG	CGTGTCGGGG	AATAGAGAAA	TCGTCTCAGA
3951	AAGCTGCGCT	GATGGTGGTG	AGAGCGGACT	GTCGCTCAGG	GGCGCCCGCG
4001	GTCTCTGCAC	CCAGGGCAGC	AGTGTGGGAT	GGCGCTGGGC	AGCCACCGCC
4051	GCCAGGAAGG	ACGTGACTCT	CCATCCTTTA	CACTTCTTTC	TCAAAGGTTT
4101	CCCGAAAGTG	CCCCCCGCCT	CGAAAACTGG	GGCCGGTGCG	GGGGGGGGGA
4151	GAGGTTAGGT	TGAAAACCAG	CTGGACACGT	CGAGTTCCTA	AGTGAGGCAA
4201	AGAGGCGGGG	TGGAGCGGGC	TCTGGAGCGG	GGGAGTCCTG	GGACTCGGTC
4251	CTCGGATGGA	CCCCGTGCAA	AGACCTGTTG	GAACAAGAGT	TGCGCTTCCG
4301	AGGTTAGAAC	AGGCCAGGCA	TCTTAGGATA	GTCAGGTCAC	CCCCCCCCCC
4351	AACCCCACCC	GAGTTGTGTT	GGTGAATTTC	TTGGAGGAAT	CTTAGCCGCG
4401	ATTCTGTAGC	TGGTGCAAAA	GGAGGAAAGG	GGTGGGGGAA	GGAAGTGGCT
4451	GTGCGGGGGT	GGCGGTGGGG	GTGGAGGTGG	TTTAAAAAGT	AAGCCAAGCC
4501	AGAGGGAGAG	GTCGAGTGCA	GGCCGAAAGC	TGTTCTCGGG	TTTGTAGACG
4551	CTTGGGATCG	CGCTTGGGGT	CTCCTTTCGT	GCCGGGTAGG	AGTTGTAAAG
4601	CCTTTGCAAC	TCTGAGATCG	TAAAAAAAAT	GTGATGCGCT	CTTTCTTTGG
4651	CGACGCCTGT	TTTGGAATCT	GTCCGGAGTT	AGAAGCTCAG	ACGTCCACCC
4701	CCCACCCCCC	GCCCACCCCC	TCTGCCTTGA	ATGGCACCGC	CGACCGGTTT
4751	CTGAAGGATC	TGCTTGGCTG	GAGCGGACGC	TGAGGTTGGC	AGACACGGTG
4801	TGGGGACTCT	GGCGGGGCTA	CTAGACAGTA	CTTCAGAAGC	CGCTCCTTCT
4851	AACTTTCCCA	CACCGCTCAA	ACCCCGACAC	CCCCGCGGCG	GACTGAGTTG
4901	GCGACGGGGT	CAGAGTCTTC	TGGCTGAAAG	TTAGATCCGC	TAGGGGTCGG
4951	CTGCCTGTCG	CTAGAAGCAT	TATTTGGCCT	CTCGGAGACC	CGTGTGGAGG
5001	AAGTGCTGGA	GTGTGCGAGT	GTGTTTGCGT	GTGTGTGTGT	GTGTGTGTGT
5051	GTGTGTGTGT	GTGTGTGTGT	GTGCGCGCGC	CCTTGGAGGG	TCCCTATGCG
5101	CTTTCCTTTT	CATGGAACGC	TGTCGTGAGG	CTTTGGTAAA	CTGTCTTTTC
5151	GGTTCCTCTC	TCGGCTGCAC	TTAAGCTTTG	TCGGCGCTGT	AAAGAGACGC
5201	GTCTTCAAGT	GCACCCTGAT	CCTCAGGCTT	CAGATAACCC	GTCCCCGAAC
5251	CTGGCCAGAT	GCATTGCACT	GCGCGCCGCA	GGTAGAGACG	TGCCCCACGT
5301	CCCCTGCGTG	CAGCGACTAC	GACCGAGAGC	CGCGCCAGTG	TGGTGTCCCG
5351	CCGAGAGTTC	CTCAGAGCAG	GCGGGGACAA	CTCCCAGACG	GCTGGGGCTC
5401	CAGCTGCGGG	CGCGGAGGTT	GGCCTCGCTC	GCAGGGGCTG	GACCCAGCCG
5451	GGGTGGGAGG	ATGGAGGAGG	GGCGGGCGGG	CTCTTCGGTG	AGTGGGGCGG
5501	GGCCTCTGGG	TCCACGTGAC	TCCTAGGGGC	TGGAAGAAAA	ACAGAGCCTG
5551	TCTGCTCCAG	AGTCTCATTA	TATCAAATAT	CATTTTAGGA	GCCATTCCGT

FIGURE 10B

5601 AGTGCCATTC GGAGCGACGC ACTGCCGCAG CTTCTCTGAG CCTTTCCAGC
5651 AAGTTTGTTC AAGATTGGCT CCCAAGAATC ATGGACTGTT ATTATGCCTT
5701 GTTTTCTGTC AGTGAGTAGA CACCTCTTCT TTCCCTTCTT GGGATTTCAC
5751 TCTGTCCTCC CATCCCTGAC CACTGTCTGT CCCTCCCGTC GGACTTCCAT
5801 TTCAGTGCCC CGCGCCCTAC TCTCAGGCAG CGCTATGGTT CTCTTTCTGG
5851 TCCCTGCAAG GCCAGACACT CGAAATGTAC GGGCTCCTTT TAAAGCGCTC
5901 CCACTGTTTT CTCTGATCCG CTGCGTTGCA AGAAAGAGGG AGCGCGAGGG
5951 ACCAAATAGA TGAAAGGTCC TCAGGTTGGG GCTGTCCCTT GAAGGGCTAA
6001 CCACTCCCTT ACCAGTCCCG ATATATCCAC TAGCCTGGGA AGGCCAGTTC
6051 CTTGCCTCAT AAAAAAAAAA AAAAAAACAA AAAACAAACA GTCGTTTGGG
6101 AACAAGACTC TTTAGTGAGC ATTTTCAACG CAGCGACCAC AATGAAATAA
6151 ATCACAAAGT CACTGGGGCA GCCCCTTGAC TCCTTTTCCC AGTCACTGGA
6201 CCTTGCTGCC CGGTCCAAGC CCTGCCGGCA CAGCTCTGTT CTCCCCTCCT
6251 CCTGTTCTTA ACCAGCTGGA AGTTGTGGAA ATTGGGCTGG AGGGCGGAGG
6301 AAGGGCGGGG GTGGGGGGGT GGAGAAGGTG GGGGGGGGGG AGGCTGAAGG
6351 TCCGAAGTGA AGAGCGATGG CATTTTAATT CTCCCTCCNC CTCCCCCCTT
6401 TACCTCCTCA ATGTTAACTG TTTATCCTTG AAGAAGCCAC GCTGAGATCA
6451 TGGCTCAGAT AGCCGTTGGG ACAGGATGGA GGCTATCTTA TTTGGGGTTA
6501 TTTGAGTGTA AACAAGTTAG ACCAAGTAAT TACAGGGCGA TTCTTACTTT
6551 CGGGCCGTGC ATGGCTGCAG CTGGTGTGTG TGTGTGTAGG GTGTGAGGGA
6601 GAAAACACAA ACTTGATCTT TCGGACCTGT TTTACATCTT GACCGTCGGT
6651 TGCTACCCCT ATATGCATAT GCAGAGACAT CTCTATTTCT CGCTATTGAT
6701 CGGTGTTTAT TTATTCTTTA ACCTTCCACC CCAACCCCCT CCCCAGAGAC
6751 ACCATGATTC CTGGTAACCG AATGCTGATG GTCGTTTTAT TATGCCAAGT
6801 CCTGCTAGGA GGCGCGAGCC ATGCTAGTTT GATACCTGAG ACCGGGAAGA
6851 AAAAAGTCGC CGAGATTCAG GGCCACGCGG GAGGACGCCG CTCAGGGCAG
6901 AGCCATGAGC TCCTGCGGGA CTTCGAGGCG ACACTTCTAC AGATGTTTGG
6951 GCTGCGCCGC CGTCCGCAGC CTAGCAAGAG CGCCGTCATT CCGGATTACA
7001 TGAGGGATCT TTACCGGCTC CAGTCTGGGG AGGAGGAGGA GGAAGAGCAG
7051 AGCCAGGGAA CCGGGCTTGA GTACCCGGAG CGTCCCGCCA GCCGAGCCAA
7101 CACTGTGAGG AGTTTCCATC ACGAAGGTCA GTTTCTGCTC TTAGTCCTGG
7151 CGGTGTAGGG TGGGGTAGAG CRCCGGGGCA GAGGGTGGGG GGTGGGCAGC
7201 TGGCAGGGCA AGCTGAAGGG GTTGTGGAAG CCCCCGGGGA AGAAGAGTTC
7251 ATGTTACATC AAAGCTCCGA GTCCTGGAGA CTGTGGAACA GGGCCTCTTA
7301 CCTTCAACTT TCCAGAGCTG CCTCTGAGGG TACTTTCTGG AGACCAAGTA
7351 GTGGTGGTGA TGGGGGAGGG GGTTACTTTG GGAGAAGCGG ACTGACACCA
7401 CTCAGACTTC TGCTACCTCC CAGTGGGTGT TCTTTAGCTA TACCAAAGTC
7451 AGGGATTCTG CCCGTTTTGT TCCAAAGCAC CTACTGAATT TAATATTACA
7501 TCTGTGTGTT TGTCAGGTTT ATCAATAGGG GCCTTGTAAT ACGATCTGAA
7551 TGTTTCCTAG CGGATGTTTC TTTTCCAAAG TAAATCTGAG TTATTAATCC
7601 TCCAGCATCA TTACTGTGTT GGAATTTATT TTCCCTTCTG TAACATGATC
7651 AACAAGGCGT GCTCTGTGTT TCTAGGATCG CTGGGGAAAT GTTTGGTAAC
7701 ATACTCAAAA GTGGAGAGGG AGAGAGGGTG GCCCCTCTTT TTCTTTACAA
7751 CCACTTGTAA AGAAAACTGT ACACAAAGCC AAGAGGGGGC TTTAAAAGGG
7801 GAGTCCAAGG GTGGTGGAGT AAAAGAGTTG ACACATGGAA ATTATTAGGC
7851 ATATAAAGGA GGTTGGGAGA TACTTTCTGT CTTTGGTGTT TGACAAATGT
7901 GAGCTAAGTT TTGCTGGTTT GCTAGCTGCT CCACAACTCT GCTCCTTCAA
7951 ATTAAAAGGC ACAGTAATTT CCTCCCCTTA GGTTTCTACT ATATAAGCAG
8001 AATTCAACCA ATTCTGCTAT TTTTTGTTTT TGTTTCTTGT TTTTGTTTTG
8051 TTTGGTTTTT TTTTTTTTTT TTTTTTTTTT GTCTCAGAAA AGCTCATGGG
8101 CCTTTTCTTT TCCCCTTTCA ACTGTGCCTA GAACATCTGG AGAACATCCC
8151 AGGGACCAGT GAGAGCTCTG CTTTTCGTTT CCTCTTCAAC CTCAGCAGCA
8201 TCCCAGAAAA TGAGGTGATC TCCTCGGCAG AGCTCCGGCT CTTTCGGGAG
8251 CAGGTGGACC AGGGCCCTGA CTGGGAACAG GGCTTCCACC GTATAAACAT
8301 TTATGAGGTT ATGAAGCCCC CAGCAGAAAT GGTTCCTGGA CACCTCATCA
8351 CACGACTACT GGACACCAGA CTAGTCCATC ACAATGTGAC ACGGTGGGAA

FIGURE 10C

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8401  ACTTTCGATG TGAGCCCTGC AGTCCTTCGC TGGACCCGGG AAAAGCAACC
8451  CAATTATGGG CTGGCCATTG AGGTGACTCA CCTCCACCAG ACACGGACCC
8501  ACCAGGGCCA GCATGTCAGA ATCAGCCGAT CGTTACCTCA AGGGAGTGGA
8551  GATTGGGCCC AACTCCGCCC CCTCCTGGTC ACTTTTGGCC ATGATGGCCG
8601  GGGCCATACC TTGACCCGCA GGAGGGCCAA ACGTAGTCCC AAGCATCACC
8651  CACAGCGGTC CAGGAAGAAG AATAAGAACT GCCGTCGCCA TTCACTATAC
8701  GTGGACTTCA GTGACGTGGG CTGGAATGAT TGGATTGTGG CCCCACCCGG
8751  CTACCAGGCC TTCTACTGCC ATGGGGACTG TCCCTTTCCA CTGGCTGATC
8801  ACCTCAACTC AACCAACCAT GCCATTGTGC AGACCCTAGT CAACTCTGTT
8851  AATTCTAGTA TCCCTAAGGC CTGTTGTGTC CCCACTGAAC TGAGTGCCAT
8901  TTCCATGTTG TACCTGGATG AGTATGACAA GGTGGTGTTG AAAAATTATC
8951  AGGAGATGGT GGTAGAGGGG TGTGGATGCC GCTGAGATCA GACAGTCCGG
9001  AGGGCGGACA CACACACACA CACACACACA CACACACACA CACACACACA
9051  CACGTTCCCA TTCAACCACC TACACATACC ACACAAACTG CTTCCCTATA
9101  GCTGGACTTT TATCTTAAAA AAAAAAAAAA GAAAGAAAGA AAGAAAGAAA
9151  GAAAAAAAAT GAAAGACAGA AAAGAAAAAA AAAACCCTAA ACAACTCACC
9201  TTGACCTTAT TTATGACTTT ACGTGCAAAT GTTTTGACCA TATTGATCAT
9251  ATTTTGACAA ATATATTTAT AACTACATAT TAAAAGAAAA TAAAATGAG
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FIGURE 10D

bmp2p

GAATTCATTTAAACT[illegible]ATTCACTTCTAGGTCCCATGCGTTTACACT[illegible]T
TTCCACCACAAGAGGGCAGCCATCTCTAAAAAAACAACAGTCGAGTGCTC
TTCAGAGAAATTGGGCCAAACTTGAGGAAAGTTCCTGGGAAAGGCTTTTT
AGCAGCACCTCTCTGGGCTACAAAAAAGAAGCCAGCAGGCACCACCAAGG
TGGAGTAACTGTCCAGAGGCATCCATTTTACCTCAGAGACTTGATTACTA
AGGATATCCTAAACGGCCAAACTCTCTCTTCTGGTGTTCCAGAGGCCCAA
AGCTGCAAGGCATTGTTGATGTCATCACCAAAGGTTTCATTTTCATCTTT
TCTTGGGGTTGGTCCAACAGCTGTCAGCTTTCTCTTCCTCATTAAAGGCA
ACTTTCTCATTTAAATCTCATATAGGTTCGGAGTTTCTTGCTTTGCTCCT
TCCGCCTCCGCGATGACAGAAGCAATGGTTAACTTCTCAATTAAACTTGA
TAGGGAAGGAAATGGCTTCAGAGGCGATCAGCCCTTTTGACTTACACACT
TACACGTCTGAGTGGAGTGTTTTATTGCCGCCTTGTTTGGTGTCTCATGA
TTCAGAGTGACAACTTCTGCAACACGTTTTAAAAAGGAATACAGTAGCTG
ATCGCAAATTGCTGGATCTATCCCTTCCTCTCCTTTAATTTCCCTTGTAG
ACAGCCTTCCTTCAAAAATACCTTATTTGACCTCTACAGCTCTAGAAACA
GCCAGGGCCTAATTTCCCTCTGTGGGTTGCTAATCCGATTTAGGTGAACG
AACCTAGAGTTATTTTAGCTCCCCGACTGAAAAGCTAGCACACGTGGGTA
AAAAAATCATTAAAGCCCCTGCTTCTGGTCTTTCTCGGTCTTTGCTTTGC
AAACTGGAAAGATCTGGTTCACAACGTAACGTTATTCACTCTGGTCTTCT
ACAGGAATGCTCAGCCCATAGTTTTGGGGGTCCTGTGGGTAGCCAGTGGT
GGTACTATGAAGGCTCCTGAATGTAGGGAGAAATGGAAAGATTTCAAAAA
AGAATCCTGGCTCAGCAGCTTTGGGGACATTTCCAGCTGAGGAAGAAAAC
TGGCTTGGCCACAGCCAGAGCCTTACTGCTGGAGACCCAGTGGAGAGAGA
GGACCAGGCAGAAAATTCAAAGGTCTCAAACCGGAATTGTCTTGTTACCT
GACTCTGGAGTAGGTGGGTGTGGAAGGGAAGATAAATATCACAAGTATCG
AAGTGATCGCTTCTATAAAGAGAATTTCTATTAACTCTCATTGTCCCCTC
ACATGGACACACACACACACACACACACACACACACACACACATCACTAGAA
GGGATGTCCACTTTACAAGTGTGTATCTATGTTCAGAAACCTGTACCCGT
ATTTTTATAATTTACATAAATAAATACATATAAAATATATGCATCTTTTT
ATTAGATTCATTTATTTGAATATAAATGTATGAATATTTATAAAATGTAA
TAATGCACTCAGATGTGTATCGGCTATTTCTCGACATTTTCTTCTCACCA
TTCAAAACAGAAGCGTTTGCTCACATTTTTGCCAAAATGTCTAATAACTT
GTAAGTTCTGTTCTTCTTTTTAATGTGCTCTTACCTAAAAACTTCAAACT
CAAGTTGAATATTGGCCCAATGAGGGAACTCAGAGGCCAGTGGACTCTGG
ATTTGCCCTAGTCTCCCGCAGCTGTGGCGCGGATCCAGGTCCCGGGGGT
CGGCTTCACACTCATCCGGGACGCGACCCCTTAGCGGCCGCGCGCTCGCC
CCGCCCCGCTCCACCGCGGCCGCCCCGTAGGGCGCGCCGTCCACACCCCT
GCGCGCCGCTCCCGCCCGCCCGGGGATCCCCGGCGCGCTGCGCCTCCGAG
GGGGAGGTGTTCGGCCACGGCCGGGAGGGAGCCGGCAGGCGGCGTCTCCT
TTAAAAGCCGCGAGCGCCGCGCCACGGCGCCTCCGTCGTCGCCGCCGGAG
TCCTCGCCCTGCCGCGCAGAGCCCTGCTCGCACTGCGCCCGCCGCCGCTG
CGCTTCCCACAGCCCGCCCGGGATTGGCAGCCCCGGACGTAGCCTCCCCA
GGCGACACCAGGCACCGGACGCCCTCCCGGCGAAAGACGCGAGGGTCACC
CGCGGCTTCGAGGGACTGGCACGACACGGGTTGGAACTCCAGACTGTGCG
CGCCTGGCGCTGTGGCCTCGGCTGTCCGGGAGAAGCTAGAGTCGCGGACC
GACGCTAAGAACCGGGAGTCCGGAGCACAGTCTTACCCTCAATGCGGGGC
CACTCTGACCCAGGAGTGAGCGCCCAAGGCGAGCGGGCGGAAGAGTGAGT
GGACCCCAGGCTGCCACAAAAGACACTTGGCCCGAGGGCTCGGAGCGCGA
GGTCACCCGGTTTGGCAACCCGAGACGCGCGGCTGGACTGTCTCGAGAAT
GAGCCCCAGGACGCCGGGGCGCCGCAGCCGTGCGGGCTCTGCTGGCGAGC
GCTGATGGGGGTGCGCCAGAGTCAGGCTGAGGGATGCAGAGTGGCGGCCC
GCCCGCCACCCAGATCTTCGCTGCGCCCTTGCCCGGACACGGCATCGCCC
ACGATGGCTGCCCCGAGCCATGGTCGCGGCCCAGCTAACGCAGAACGTC
CGTCCCTCGCCCGGCGAGTCCCGGAGCCAGCCCCGCGCCCCGCCAGCGCT
GGTCCCTGAGGCCGACGACAGCAGCAGCCTTGCCTCAGCCTTCCCTTCCC
GTCCCGGCCCCGCACTCCTCCCCCTGCTCGAGGCTGTGTGTCAGCACTTG
GCTGGAGACTTCTTGAACTTGCCGGGAGAGTGACTTGGGCTCCCCACTTC
GCGCCGGTGTCCTCGCCCGGCGGATCC

Figure 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/08197

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12Q 1/68; C07H 21/04; C12N 15/09
US CL :435/6, 172.3, 320.1; 536/23.1, 24.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 172.3, 320.1; 536/23.1, 24.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, EMBASE, BIOSIS, CAPLUS, SCISEARCH, WPIDS
search terms: bone morphogenic, osteogen?, DNA, nucleic, gene#, BMP-2A, BMP-2B, BMP-2, BMP-4, Feng J, Harris S

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,166,058 A (WANG et al.) 24 November 1992, columns 1-2.	1-4, 6-10
Y	WO 92/13091 A1 (ONCOGENE SCIENCE, INC.) 06 August 1991, pages 27-31.	1-4, 6-10
X	GHOSH-CHOUDHURY et al. Expression of the BMP 2 gene during bone cell differentiation. Critical Reiews in Eukaryotic Gene Expression. 1994, Vol. 4, No. 2 & 3, pages 345-355, especially pages 349-353.	1-4, 6-10
X ----- Y	KURIHARA et al. Murine bone morphogenic protein 4 gene: Existence of multiple promoters and exons for the 5'-untranslated region. Biochem. Biophys. Res. Commun. 14 May 1993, Vol. 192, No. 3, pages 1049-1056, especially page 1053.	6, 7 -------- 1-4, 8-10

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 SEPTEMBER 1996	11 OCT 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer SCOTT D. PRIEBE Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/08197

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	FENG et al. Structure and sequence of mouse bone morphogenic protein-2 gene (BMP-2): Comparison of the structures and promoter regions of BMP-2 and BMP-4 genes. Biochim. Biophys. Acta. 21 June 1994, Vol. 1218, pages 221-224.	6, 7 --- 1-4, 8-10
X	HARRIS et al. Development of osteoblast cell lines from transgenic mice containing bone mporphogenic protein 2 (BMP2) promoter-T-antigen constructs: Analysis of BMP 2 retinoic acid and 1,25 (OH)2 vitamin D response regions in the BMP 2 promoter in the context of chromatin structure. J. Cell. Biochem. February 1994, Supplement O (18B), page 392.	1-4, 6-10
X --- Y	HARRIS et al. Retinoid regulation of bone morphogenic protein 4 (BMP 4 or DVR 4): Analysis of the mouse BMP 4 gene promoter by transfection into primary cultures of fetal rat calvariae (FC) osteoblasts. J. Cell. Biochem. 1993, Supplement O (17 Part D), page 159.	1-3, 6-10 --- 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/08197

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.